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FOREWORD

THE GENESIS OF A NEW SCIENCE,—BACTERIOLOGY

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"Die Bakteriologie ist ein Kind der jüngsten Zeit."—FRAENKEL, 1886.

Sciences are not made but born, and lineage often sheds light upon development. It was an acute observation of the late C. S. Peirce that some of the most fruitful of modern sciences have been bred by the crossing of older ones. Mathematical astronomy, physical chemistry, physiological psychology, astrophysics are examples, and the same thing is true of the applied sciences, as witness electrical, chemical and sanitary engineering; bio-chemistry; electro-chemistry.

Bacteriology is the offspring of microscopical science hybridized with the art of bacterial cultivation,—in other words, of microscopy and bacterial horticulture. The compound microscope was invented and bacteria and other micro-organisms were observed in the seventeenth century but no great progress was made in our knowledge of microbes—with the single exception of yeast—until methods for their selective cultivation or breeding similar to those long practised in agriculture and horticulture were discovered and introduced by Pasteur, Lister and Koch. In his studies in zymology and his long and arduous battle against spontaneous generation, Pasteur became proficient in "sterilizing" nutrient liquid soils or "substrata" which he afterwards planted or "inoculated" with "traces" of micro-organisms. These traces after incubation and multiplication produced overgrowths if not pure cultures of particular kinds of micro-organisms, in manageable quantities sufficient for reasonably thorough examination. In this way, Pasteur

was able to magnify microscopic into macroscopic characteristics, and for the first time made it practicable to differentiate and classify bacteria with some accuracy. It should also be remembered that Pasteur's disciple and follower, Joseph Lister, made improvements in the method of pure culture by "dilution" during his studies upon milk and the lactic fermentation.

Pasteur, very early in his work, had insisted upon the indispensability of the microscope in all investigations of yeasts and other microbes, as well as in fermentations, putrefactions and diseases (of wine and beer), which they produce, and only those who have taken the trouble to read the preposterous paper in which Liebig, the most eminent chemist and fermentation expert of his day, ridicules the use of the microscope—a paper which Huxley has rightly pronounced the most surprising that ever appeared in a sober scientific journal—can appreciate the immense service done by Pasteur in developing the microscope as an instrument of research. It was his insistence upon the use of the microscope superadded to a rigid and refined technique all along the line which enabled him to win one of the hardest fought and most important scientific battles of the nineteenth century, namely, that on behalf of biogenesis.

Pasteur is thus at once the pioneer and the founder of that wonderful science of which the present new JOURNAL is to stand as an American exponent.

But it is very doubtful if bacteriology would ever have attained even a tithe of its present development and importance if the methods of Pasteur and Lister had not been supplemented and largely displaced by those of Robert Koch, who is at once the protagonist of the new science and the architect of that imposing superstructure now known as bacteriology, built chiefly since 1881 by Koch himself and his pupils upon the foundations laid by Pasteur.

In his earlier work, Koch employed substantially the methods of liquid culture of Pasteur and Lister, but before long he vastly improved upon these cultures by thickening them with gelatine or agar—a step to which he was led through the use of potato an opaque medium for which it was obviously desirable to sub-

stitute something transparent. Brefeld and other botanists had already used gelatine, for "gelatine was first employed by Vittadini in 1852 in the culture of microscopic Fungi and has been frequently used since that time, especially by Brefeld. Klebs more recently, in 1873, recommends it especially for the cultivation of bacteria." (DeBary, 1886).

It was in 1881 that the gelatine-tube culture method and in 1883 that the gelatine-plate culture method were introduced by Koch—his first great work, namely, that upon anthrax, and also that on typhoid fever, having been done before this time, and his well known "rules," "postulates" or "laws" having also been laid down before his perfection, if not before his invention, of the solid-culture method.

The word "bacteriology" had appeared before 1886 but the subject had no existence anywhere much before that time and very little for a year or two later. In the United States, bacteriology was hardly heard of before 1885 but by 1890 it had become well known. Unfortunately, neither Great Britain nor America can make any claim to the earliest work. The terms "sterilization" and "cultivation," in our modern sense, and the word "microbe," were introduced by Pasteur and his school. The terms "pure culture," "colony," "gelatine," "agar," the use of the oil immersion objective, and the art of dyeing microscopic specimens of bacteria, come from Koch and the German school. Bacteriology is now, however, very widely cultivated both in Great Britain and the United States, and it is fitting that a JOURNAL OF BACTERIOLOGY should be published in the English language in honor of the thirty or more years of service which one of the broadest and most fundamental of the biological sciences may now claim.

Because of the intensely practical bearings of bacteriology upon medicine, and especially because of the marvellous surgical and pathological discoveries which were the first, and must ever remain the greatest, fruits of bacteriology, its botanical, agricultural, sanitary, industrial, household and economic importance were at first obscured and neglected. But of recent years these have rapidly become clear and even conspicuous, and today

hardly any field of the vast domain of the parent science, biology, is more esteemed or more cultivated than is bacteriology. Nor is this strange for, thanks to the microscope and the methods of bacteriology, the microscopic world, of which almost nothing was known when Pasteur began his researches, stands before us today in a revelation of extent, variety, magnitude and interest second only in importance and impressiveness to that other and distant world which has been revealed by the telescope and the methods of astronomy. The region of the "infinitely little" seems, however, even more directly and intimately connected with our everyday life than that remoter world. The microscopic world, indeed, lacks the majesty and grandeur of that telescopic world which lies so far beyond our reach and overawes us with sheer distance, heat, light and immensity. But the revelations of the microscope and the lessons of bacteriology have so direct, so intimate, and so fateful association with almost every aspect of the conduct of our daily and personal life—with food and drink, with health and disease, with life and death even—that they gain in intimacy what they lose in grandeur.

Bacteriology must henceforward be recognized as a broad and fundamental branch of science, coördinate with, rather than subordinate to, the other grand divisions of biology such as medicine, agriculture, zoölogy and botany. It is today of immense theoretical and practical importance, and it bids fair to become vastly greater and more important tomorrow. Hence the obvious desirability of a JOURNAL which shall cover the whole field and be devoted to the subject in its broadest aspects.

The time has forever gone by when bacteriology can be regarded merely, or even chiefly, as the handmaid of medicine or pathology. It is no less the servant of agriculture, of industry, of sanitation and of household life. It is already important in sanitary engineering, and indispensable in the arts of food production and food conservation. In its further differentiation and development the present JOURNAL should be a powerful factor. May the event justify both our hope and our expectation.

Massachusetts Institute of Technology
Boston, February, 1916

THE PEDAGOGICS OF BACTERIOLOGY[1]

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I propose to consider the subject of teaching bacteriology from several standpoints, and especially the place of bacteriology in scientific education and in medical education.

The students who, in the past, have demanded a knowledge of bacteriology as a part of their instruction in general biology have been much fewer than the number that should be seeking this knowledge; in fact the demand has been really insignificant in comparison with the importance of the study. An explanation for the neglect of bacteriology as a part of the general training of students of the biological sciences is difficult to find, but it is evident that the teachers have been to blame, chiefly because they have failed to emphasize the importance of the study from an educational standpoint. They have been interested, more in the practical application of a knowledge of bacteriology, than in the development of the educational importance of the subject.

The aim in modern education is to train the individual for usefulness. With the present crowded curricula in schools and colleges it is essential that the material presented for the training of students be selected with the greatest care. Each study should be carefully weighed in order to determine its educational value. It is necessary not only to select the subjects to be taught, but also to arrange the order in which they may be presented so as to obtain the greatest benefit from each.

In the modern organization of society the interests of different callings are so diverse as to call for general as well as special training. This fact is now recognized in the preliminary education

[1] Presidential address, Seventeenth Annual Meeting, Society of American Bacteriologists, Urbana, Ill., December 28, 1915.

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demanded of those who expect to enter the various professions. There is a special need for broad general education in science for all persons who wish to be equipped for the most efficient service to mankind.   The aim of education should be, not merely to give information, but to indicate how that information should be used, and only in so far as education aids in the promotion of the general welfare, does it meet the ideal.   The extent of the training of each individual must depend upon his ability to receive and apply the knowledge which is being disseminated in educational institutions.

A particular science may be studied from two principal aspects, namely, the practical application of the knowledge gained to the solution of problems in a special field, and the educational value of a knowledge of the science in broadening one's concept of the various forces and agencies in nature.

The science of bacteriology has extended its ramifications in so many directions that its study has become of interest and direct value to the student in many fields.   A knowledge of bacteriology enters in a prominent way into most of the activities of mankind, and for this reason it should receive much wider recognition as a subject for general educational training than it is receiving today.   The educational value of the study of bacteriology has received recognition slowly and for a study of such immense practical importance it has been taken up, for its educational value, by a comparatively small number of students in our colleges and universities.   Yet there are few subjects taught that touch upon so many phases of man's activities or so many of the conditions influencing his environment as does a course in bacteriology, and, it is safe to say, few other subjects can have greater educational value.   No one can fully appreciate the relation of bacteria to many vital problems without having studied the subject at first hand.   It is only by seeing the activities of the bacteria in the test tube, under diverse conditions, that one can gain an insight into their prominent place in many biological processes.

The relation of the bacteria to the nitrogen cycle in nature is most illuminating to the student.   The function of the bacteria

in the decomposition of organic matter as they work over the useless constituents of dead plants and animals into forms in which they may be utilized as food by the higher plants is of the greatest importance in nature. The control and purposeful utilization of this same function of the bacteria in the preparation and preservation of food materials for man and animals, and the relation of the bacteria to water and sewage purification, are examples of the regulation of bacterial action for the economic and hygienic advantage of the human race. Of equal significance are the utilizations of the functions of the bacteria in agriculture, in domestic science and the industries; and of even greater importance are the methods of controlling the action of the bacteria in their relations to sanitary science and clinical medicine.

The earliest practical application of bacteriology was to the fermentation industries through the investigations of Louis Pasteur. This was soon followed by his pioneer work in diseases of animals, especially chicken cholera and anthrax. In this latter field Pasteur laid the foundations for our later work in immunology and protective inoculations while the studies of Robert Koch paved the way for the application of bacteriology to the solution of problems in the etiology of disease and in sanitary science.

The earliest demand for a knowledge of bacteriology came from the medical profession, concerning the activities of the pathogenic bacteria, and the first courses were given to graduates in medicine. These were followed later by courses for undergraduate students of medicine, of dentistry, and of veterinary medicine. The extension of our knowledge of the activities of bacteria in nature, in fields other than disease production, soon led to the development of courses for the sanitarian, the student of dairying, and the student of agriculture. In all these courses chief stress was laid upon the practical application of the knowledge gained to the solution of problems arising in these different fields. The concentration of endeavor and interest along such lines has yielded a great fund of knowledge which is now being utilized in enhancing the welfare of man.

In recent years there has been a slowly growing demand for a

knowledge of bacteriology on the part of students in biology, especially by seniors in arts and sciences, and by graduate students. The chief interest of this group of students is to gain a broader insight into the relations of the bacteria to many of the important problems of life. Some of these students are preparing to teach biology, and others are already teaching in high schools and colleges.

While in the earlier courses, offered to graduates and undergraduates in medicine, the subject matter presented was intended largely to facilitate the application of the knowledge gained to practical questions in medicine and sanitary science, the courses for students in the arts and sciences have taken on a somewhat different aspect. For these students it has been deemed preferable to lay greater stress upon the broad fundamental biological principles involved and much less emphasis upon the practical application of the knowledge. The students of chemistry and biology in the graduate school, and in the senior class of the course in arts and sciences, have greater interest in the general information obtainable from the study of bacteriology, than in the more intricate problems of infection and immunity, which are of primary interest to the medical student. For this reason the general course in bacteriology for science students should be developed so as to acquaint them with the relations of the bacteria to problems of food production and conservation and to problems in domestic and sanitary science.

Bacteriology can be studied with greatest profit by students in their junior and senior years in college, or by graduate students, after they have had a broad training in biology, chemistry, physics and the languages. The student of bacteriology should have had instruction in general botany and zoölogy, in plant and animal physiology, in general inorganic and organic chemistry as well as in elementary physics. With a knowledge of these subjects he will be in a position to understand something of the biological relation of the bacteria to the welfare of man and especially to the problems of sanitary science. The broadening of the preliminary education of the medical student so as to include chemistry, biology, physics and the modern languages has

made it possible to place the teaching of bacteriology to these students on a much higher plane than was formerly attainable. Their understanding of the far-reaching activities of the bacteria has thereby been greatly increased and their application of the knowledge gained, to the solution of the problems which confront them as practitioners of medicine, is already showing abundant fruit in the more intelligent attitude which medical men are assuming toward questions relating to the public health.

The student who takes up the study of bacteriology as a part of his education in the biological sciences should possess a preliminary training equal to that required of medical students. With this broader foundation it becomes possible for the teacher to present the subject in a more philosophical way, and the general training which the student receives is correspondingly made more beneficial.

The amount of instruction in bacteriology offered to science students must vary with the time available for the study and with the general and professional training which the individual student is seeking. The minimum course should be one of twelve hours a week for one semester and should be devoted to general bacteriology. After the student has acquired some knowledge of bacteriological technique and of the general characters of the bacteria, attention should be directed to the activities of the bacteria in decomposition, in fermentation, in water and sewage purification, in the dairy industries and in food production and preservation. For students who desire more profound knowledge along the various lines of general and applied bacteriology, more detailed courses should be arranged to meet their special needs, the course to be given depending in part on the application which the students desire to make of the knowledge they are seeking.

The best course of study in bacteriology for the student in biology or general science has not as yet been developed. For students beginning the study a combined lecture, laboratory, and seminar course seems to give satisfactory results. The lecture should be largely a part of the laboratory exercises and should consist in explanatory remarks preceding each new phase

of the subject that is taken up. It is desirable to give the student a brief explanation as to what he is expected to do or see and how he is to proceed in conducting the laboratory exercises. When the student has carried out a series of laboratory exercises the subject may be developed on a broader plane by a lecture emphasizing the importance of the observations made and their relation to other aspects of the study.

The almost infinite number of ways in which the bacteria and their activities react upon human life, especially in their relation to the production of disease in plants and animals, and their relation to the various industrial activities, particularly in food production and food preservation, give us inexhaustible material for study in the classroom. The knowledge which the student of bacteriology gains is of such great personal interest and importance that he is easily carried along, step by step, from simple observations to the most complex and vital phenomena of life.

The study of bacteriology serves unusually well for training the powers of observation and judgment. Every lesson is per se an object lesson and one in which the student is not only the observer, but, the demonstrator as well. Moreover the remarkable susceptibility of the bacteria to environmental influences will permit of each demonstration being modified in a variety of ways. This possibility of varying the demonstrations precludes the probability of a loss of interest on the part of the student.

It will be profitable to begin a course in general bacteriology with exercises in staining various types of bacteria. The student should record his results briefly and amplify the record with line drawings of the organisms and cultures studied. In this way he acquires some knowledge of the relative size, grouping, staining reactions, and rapidity of growth of the bacteria. The next step may be the isolation of bacteria in pure culture from mixtures and the cultivation of several species of these pure strains upon the common media employed for this purpose. In the systematic study of a culture the student may follow the general plan of description as contained in the Society card. This will acquaint him with the vocabulary generally employed in this work and will help him to recognize some of the ac-

tivities of the bacteria. After he has become familiar with bacteriological technique and the methods of studying individual cultures, he should be given pure cultures of the common types of bacteria. It is desirable that he should be able to recognize all the ordinary bacteria that may be encountered later in his work as contaminations so as to be able to avoid confusing them with other bacteria that may be of importance in the study that he is conducting. With the foregoing exercises as a foundation the student is prepared to take up the study of the bacteria in water, soil, air, milk, butter, cheese; in the different orifices of the body; and in the excretions from the body. In these studies it will be possible to observe merely a few of the more common bacteria encountered, but each phase of the subject can be amplified by lectures, assigned readings, and discussions in the seminar. In the foregoing studies special exercises may be arranged to enable the student to comprehend the relation of bacteria to decomposition, putrefaction, fermentation, nitrification, denitrification and nitrogen fixation, or these phenomena may be independently attacked after the more common bacterial flora in nature have been studied. If the latter course is pursued the relation of the bacteria to these processes should be taken up briefly as phases of the phenomena present themselves, while the detailed study of the phenomena is carried out later.

A general course in bacteriology is not complete unless the student is given at least a brief introduction to the relation of bacteria to the diseases of plants and animals. This study should include the methods of recognizing the causative agents of disease, the manner in which they produce disease, and the ways in which recovery from infection occurs. The student should also have an introduction to the bacteriological side of important problems in preventive medicine, especially the efficiency of disinfection by the use of chemicals, heat and light. The relation of bacteria to the purification of water and sewage, and to the preservation of milk, eggs, meat and vegetables should be developed by lectures, assigned readings and exercises in the laboratory.

The position of bacteriology in the curriculum in some medical schools is unsatisfactory, especially where bacteriology is taught to first year students.   It is largely a waste of time to attempt to teach clinical bacteriology to a student who knows nothing about disease in general and is not expected to take up the study of clinical medicine until one or two years later.   The difficulty can be overcome in large part by requiring that the student receive a course of instruction in general bacteriology as a part of his premedical training and then receive instruction in clinical bacteriology during the second semester of the second year, or the first semester of the third year of his medical course.   In the present arrangement of the curriculum of the medical school, if bacteriology is taught entirely in the first year, the student has usually not completed the study of physiology nor has he, as a rule, begun to study pathology and clinical medicine.   The anomalous position of bacteriology in the medical curriculum is probably due to the fact that those responsible for the condition fail to appreciate the broad biological relations of the science of bacteriology.

The student of clinical bacteriology who lacks a knowledge of physiology, of pathology, and of clinical medicine, suffers a serious handicap in appreciating the principles that underlie the pathogenic action of the bacteria and the reactions of the body to infection.   The problems of infection and immunity have the most important relations to normal and abnormal conditions of the body and these relations cannot be fully comprehended without a knowledge of physiology and of pathology.

Many of the colleges that prepare students for the medical course could be equipped, without great expense, if not already prepared to do so, to give a course in elementary bacteriology in their biological departments through teachers of those departments who would develop the subject on a broad biological basis. With such a preliminary training in general bacteriology the medical student could then take up clinical bacteriology with much greater profit in the second or third year of his medical course because he could appreciate the relation of the subjects to their clinical application.

If general bacteriology is placed in the premedical course it will be necessary to lengthen that course to three years, at least. This would involve no hardship for those students taking a combined science and medical course in seven years, nor for the students entering a medical school that requires a college degree as a requisite for entrance to the medical course. This plan would also afford opportunity to extend the premedical course so as to include organic chemistry and a broader training in biology and the modern languages, as many students enter the medical school with insufficient preparation in these three subjects.

In the second half of the second year or the beginning of the third year of the medical course, the student should have completed his studies in physiology and have had a course in general pathology. He would then pursue his studies in clinical bacteriology with much greater intelligence and profit.

The course in bacteriology adapted to the needs of medical students should consist, at present, of preliminary work in the acquirement of technique, the ability to isolate and recognize individual species of bacteria, the study of the common saprophytic bacteria and their important functions in nature, especially their relation to decomposition, putrefaction and fermentation, and the utilization of the functions of the bacteria in the purification of water and sewage. As persons with a broad scientific training, graduates in medicine should have as deep an insight into all of the foregoing activities of the bacteria as it is possible to give them.

With this fundamental knowledge the medical student is in a position to comprehend more fully the relation of the bacteria to disease, and the various measures which are employed by sanitarians to combat and eradicate disease.

The more practical side of the training of medical students will deal with the recognition of the pathogenic bacteria, a knowledge of the effects which they produce in the body in causing disease, and the reactions of the body in overcoming the disease.

The extent to which the medical student should be trained in the various phases of clinical bacteriology cannot be stated

categorically, but it may be emphasized that the more detailed the laboratory studies in infection and immunity, the greater the assistance to the student in obtaining a grasp of the subject and hence the more intelligent the application which he will make of the knowledge obtained, to the problems in clinical medicine and therapeutics.

If the medical student were to receive instruction in general bacteriology in his premedical course, it would be possible to devote more time to clinical bacteriology and its application to the diagnosis, treatment and prevention of disease during his medical course. Colleges and universities should therefore be equipped to give courses in general and special bacteriology to students in the premedical, the arts and sciences, and the sanitary engineering courses, as well as to science students in the graduate school. Besides courses in general bacteriology, more advanced courses should be offered, especially in the bacteriology of water and sewage, in dairy bacteriology, in agricultural bacteriology, in domestic science bacteriology and in sanitary science bacteriology.

It is evident that if the knowledge to be gained through a course in general bacteriology were more widely diffused amongst persons in all walks of life, there would be far less credence given to the extravagant and false claims of the horde of quacks and faddists who are now preying upon an ignorant and credulous public. The light of truth alone can relieve us of the depredations of those who claim to practice those "isms" that have been raised up because of the general ignorance of mankind.

In order to further the development of bacteriology and to extend the teaching of the subject to students of the biological sciences, it would be desirable for this Society to organize a teaching section for the discussion of problems in the teaching of bacteriology at each annual session. Through the interchange of views and the discussion of the principles of teaching the subject, the science of bacteriology, as well as education in general, would reap great benefit.

FURTHER STUDIES ON BACTERIAL NUTRITION: THE UTILIZATION OF PROTEID AND NON-PROTEID NITROGEN

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The highly interesting observation of Bainbridge (1911) that certain aerobic and facultative anaerobic bacteria of the gelatin-liquefying and non-liquefying types are of themselves unable to initiate decomposition of purified native proteins has been fully corroborated by Sperry and Rettger (1915). The last-named authors have shown further that the putrefactive anaerobes *B. putrificus*, *B. oedematis* (*B. oedematis-maligni*, Zopf) and *B. Feseri* (*B. anthracis-symptomatici*, Kruse) are likewise devoid of this property; and that the vegetable protein edestin, like egg and serum albumin, does not undergo disintegration by direct bacterial action. It was but natural to assume, therefore, that the protein nitrogen cannot be utilized by bacteria unless it is first simplified and made available for cell nutrition through the action of a proteolytic enzyme, strong acid or alkali, or some other cleavage-producing agent.

Solutions of purified proteins were prepared by the methods now used in all biochemical laboratories and involving the crystallization of the proteins. The test media were usually the same as those employed by Bainbridge, and contained the following ingredients, besides the protein; sodium chloride 0.5 per cent, sodium sulphate 0.2 per cent, calcium chloride 0.1 per cent and acid potassium phosphate 0.1 per cent. The only possible source of nitrogen was the protein, except in certain check tests in which small amounts of peptone were employed. The solutions containing the purified proteins were sterilized by filtration through the laboratory Berkefeld.

The test media were inoculated from 24 hour slant agar cultures of the various organisms, with the special precaution of

introducing only a small number of the bacteria and as little extraneous matter as possible. The fate of the bacteria and of the proteins was determined in three ways; first, by the plate method of determining the numbers of cultivable bacteria at the beginning of the experiments and after varying intervals or periods of incubation; second, by noting any change in the appearance of the media; and finally by determining the amount of coagulable protein at different times, during the course of the experiments.

The results obtained by Sperry and Rettger (1915) were so definite and consistent as to leave no doubt as to their significance. It was assumed that the purified proteins resisted decomposition by direct bacterial action because of their original or unchanged condition as native proteins; hence, sterilization by heat was to be avoided, as heating at coagulation temperature undoubtedly causes changes in the protein molecule.

The present investigation is in part a continuation of the work of Sperry and Rettger on the action of bacteria on purified proteins. Instead, however, of studying the behavior of bacteria toward unchanged (unheated) proteins, the experiments were conducted on test media containing coagulated egg albumin as the only possible source of nitrogen. The investigation also included a study of the behavior of bacteria toward proteoses and peptones, and of so-called "bacterial autolysis."

I. THE BEHAVIOR OF BACTERIA TOWARD PURIFIED COAGULATED EGG ALBUMIN

The egg albumin was prepared by the method of Hopkins and Pinkus (1899). The test medium containing the albumin and inorganic salts was the same as that used by Bainbridge and by Sperry and Rettger, with the exception that the medium was sterilized by heat and hence contained *coagulated* albumin. The methods of inoculation, incubation and determination of results were the same as those described in the earlier paper from this laboratory, (Sperry and Rettger, 1915). Special attention was given to the enumeration of bacteria by the usual plate method

immediately after inoculation and after definite periods of incubation at 30°C. The results are given in the accompanying Tables I, and II.

With very few exceptions, the results show that there was little multiplication of the bacteria with which the medium was inoculated. An increase of 100 per cent, or even 1000 per cent,

TABLE I

Enumeration of bacteria in inoculated test medium containing heated (coagulated) egg albumin.   Counts per cubic centimeter of medium

ORGANISMS	IMMEDIATELY	24 HOURS	48 HOURS	72 HOURS	4–7 DAYS	2 WEEKS	3 WEEKS	4 WEEKS AFTER INOCULATION
Prot. vulgaris I	2,600	3,752	2,350			4,800		
Prot. vulgaris II....	1,150	4,450	5,700			XX		
Prot. vulgaris III...	146	3,900			2,800			1,775
Prot. vulgaris IV...	358	1,650		4,032	1,070	3,520	3,840	
Prot. vulgaris V....	800	3,680	3,136			4,990	3,700	
Prot. mirabilis......	480			1,980		3,000	544	1,180
B. subtilis..........	1		1,470			XX		XX
B. prodigiosus......	640		XX			XX		XX
Staph. aureus II....	1,400	2,500	2,150					
Staph. aureus II....	3,800	4,800	7,450					
Staph. aureus III...	50	2,100			1,700			406
Staph. aureus IV...	365	864		990	860	134	75	
Staph. aureus V....	204	800	865			84	620	
Staph. aureus VI...	150				640	160	89	53
B. coli I............	17				2,000	1,200	600	675
B. coli II...........	352			2,300		2,000	260	350
B. coli III..........	1			3,800		4,700	6,100	7,200
B. typhi I..........	288		705			480		162
B. typhi II........	165			191		768	832	

NOTE: XX indicates too many colonies on the agar plates to count.

* Dilutions of 1 : 10,000 were employed in these tests.

in the numbers of organisms would not be unexpected even in what may be termed a nitrogen-free medium which is constantly exposed to the atmosphere.   In the tests with *B. prodigiosus, B. subtilis* and one of the *Proteus vulgaris* strains the numbers of colonies on the agar plates became too numerous to count. Furthermore, there were visible indications that the protein was undergoing disintegration.   These results are, therefore, in strik-

ing contrast to the rest.   The most plausible explanation of the proteolytic action in these tubes is that these organisms produced a very active proteolytic enzyme early in the course of their growth on the slant agar, so that sufficient enzyme was introduced into the test medium along with the bacteria to bring about cleavage of the albumin and thus prepare it for nitrogen assimilation by the bacteria.   These tests are being repeated.

In all of the experiments except the three just commented upon there was no visible indication of bacterial disintegration

TABLE II

Control experiments.   Enumeration of bacteria in inoculated medium containing heated (coagulated) egg albumin and 1 per cent of peptone

ORGANISMS	IMMEDI-ATELY	24 HOURS	48 HOURS	72 HOURS	4-7 DAYS	2 WEEKS	3 WEEKS	4 WEEKS AFTER INOCULA-TION
Prot. vulgaris I	11	17,300			XX			
Prot. vulgaris II....	1,472	XX		XX				
Prot. vulgaris III...	46	21,000+						
Prot. vulgaris IV...	1				XX			
B. subtilis..........	2		1,900			XX		XX
Staph. aureus I.....	680	XX		XX				
Staph. aureus II....	94	13,000						
Staph. aureus III...	1				XX			
B. coli	1				XX			
B. typhi............	256		XX					

NOTE: XX indicates too many plate colonies to count.

In all of the experiments recorded in tables I and II the test medium contained the following inorganic salts: Sodium chloride 0.5 per cent, sodium sulphate 0.2 per cent, calcium chloride 0.1 per cent, and acid potassium phosphate 0.1 per cent.

of the egg albumin.   In fact, the liquid portion of the medium remained clear and colorless, and the medium could not be distinguished from the uninoculated tubes, either by its appearance to the naked eye or by the odor.   Control tubes containing the same ingredients plus 1 per cent peptone rapidly underwent marked change.   The protein became involved and, in the tubes containing gelatin-liquefying organisms, was gradually digested.   In every instance the liquid part of the medium soon became turbid, and frequently more or less colored (see Table II).

Tests were also conducted with anaerobes of the type of *B. putrificus* and *B. oedematis*, which are characterized by their strong proteolytic and putrefactive properties. No quantitative bacterial determinations were attempted with these anaerobes, however, and the observations were confined to a study of the physical characters of the medium. No change whatever could be noted in the medium; the clear liquid and the coagulated albumin remaining apparently unaffected even after three to four weeks of incubation. Inoculation of egg-meat medium from these tubes with the aid of a platinum loop clearly demonstrated the presence of putrefactive anaerobes by the rapid and characteristic decomposition which took place in the standard egg-meat medium.

II. THE BEHAVIOR OF BACTERIA TOWARD PROTEOSES AND PEPTONES

In text books and other bacteriological publications the assumption is made that proteoses and peptones are readily attacked by all known bacteria which are easily cultivated on artificial media. So firmly has "peptone" established itself as an important ingredient of the common and standard bacteriological laboratory media that its value as the source of nitrogen supply in the cell metabolism of bacteria is taken as a matter of course. It is true that meat extract which is practically protein-free is also looked upon as practically indispensable, but not because it furnishes food as such to the organisms. By many at least it is regarded as a stimulator of cell metabolism, due to the various extractives present.

It is one of the objects of this paper to show that proteoses and peptones follow essentially the same law of resistance to direct bacterial action as do the native proteins, egg albumin, serum albumin and edestin. While the scope of the investigation has as yet been somewhat limited, sufficient data appear to us to have been acquired to warrant their publication at this time.

It is a well-known fact that the proteoses and peptones resulting from the action of proteolytic agents like pepsin and trypsin upon native proteins, and indeed all proteoses and peptones, have

thus far resisted all attempts to isolate or purify them. Hence, it has been impossible to employ all the methods of investigation in a study of their bacteriological-chemical relations which are applicable in connection with certain albumins, as for instance egg albumin. Peptones are now regarded as amino acid combinations of varying complexities, rather than proteins. Witte's peptone, which is essentially a mixture of albumoses and peptones, is far from being made up purely of these nitrogen complexes, although it has long been regarded as the standard for bacteriological purposes. The various American brands are undoubtedly even less pure than the Witte product. It does not follow, however, that they are of correspondingly less value as food for bacteria.

In our study of the behavior of various types of bacteria towards proteoses and peptones the Biuret test for proteins has been employed to great advantage. The method which has been advocated and used by Vernon (1904) for the quantitative estimation of peptone has, with slight modifications been employed by us in the present investigation and in the experiments on bacterial autolysis. A brief description of this method is given here.

The tests were made in Nessler tubes. One cubic centimeter of the inoculated culture fluid was added to 20 cc. of a 4 per cent solution of sodium hydroxide and 2 cc. of a centinormal solution of copper sulphate. To the same mixture of alkali and copper sulphate in a second tube a standard solution (0.25 per cent) of Witte's peptone was added until the same degree of color was produced as in the test medium. The quantity of peptone required in matching the colors was taken as a measure of the amount of peptone present in the inoculated and incubated culture fluid. For example, if 1 cc. of standard peptone solution was required the value of the biuret test was recorded as 1.0, since both liquids gave the same color in the same concentration. Test fluids and controls contained the same amount of Witte's peptone at the outset as the standards, namely 0.25 per cent.

Peptone solutions containing from 0.2 to 2.0 per cent of Witte's peptone were at first employed as culture media for the different

organisms, but it was soon found that the amount of peptone present should not exceed 0.25 per cent.   When the peptone was used in higher concentration slight reductions in the amount of the proteins could not be detected, or at least could not be determined accurately.   In the lower dilutions, however, the various degrees of decomposition were easily observed.

For the autolysis experiments standard peptone solutions were frequently employed for color comparison, while the work on the relation of the growth of different bacteria to proteoses and peptones involved the employment of the standard solution only as a check or control for the inoculated flasks.   The results are not given in per cent, but are represented in the tables by 0, X, XX, XXX and XXXX.   The first of these symbols, 0, indicates no reduction of the proteoses and peptones, as compared with the controls, X a slight decomposition, XX fair, XXX strong, and XXXX complete reduction of these soluble proteins.   Besides the "peptone" the test media often contained other agents, as will be seen in the tables, namely ammonium sulphate, beef extract and glucose.   Furthermore, all of the fluids contained 0.5 per cent of sodium chloride.

The results require but little comment.   With few exceptions, no disappearance of albumoses and peptones could be noted in flasks which were inoculated with members of the colon-typhoid group of organisms, even after four weeks of incubation.   In the flasks showing a reduction of the biuret reaction the apparent loss of the soluble proteins was slight, and may be accounted for by other factors than an actual decomposition by the bacteria with which they were inoculated.   In all of these experiments the bacterial growths were fairly luxuriant, particularly when the test medium contained beef extract or ammonium sulphate. Even in those instances in which slight reduction of the soluble proteins was recorded, at least two weeks, and as a rule three weeks or more, were required to show the apparent reduction.

The above experiments are being repeated.   Similar tests are also being made with media containing peptone and the ingredients of the Uschinsky medium, with soluble purified casein, or nutrose and with dialyzed proteoses.   Thus far results similar

TABLE III

Showing the behavior of certain gelatin-non-liquefying bacteria towards Witte's peptone

ORGANISMS	MEDIA	DECOMPOSITION OF PROTEOSES AND PEPTONES							
		At 37° C.				At 20° C.			
		1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks	4 weeks
B. coli	2 per cent peptone	0	0	0		0	0	0	0
	0.5 per cent peptone	0	0	0	0	0	0	0	0
	0.25 per cent peptone	0	0	0	0	0	0	0	0
	0.25 per cent peptone	0	0	0	0	0	0	0	0
B. coli (H).	0.25 per cent peptone 0.5 per cent beef extract	0	0	X	X		X?	0	
	0.25 per cent peptone 1.0 per cent glucose	0		0	X0	0		0	0
	0.25 per cent peptone 0.25 per cent ammonium sulphate		0	0	0		X	X	X
B. coli (U).	0.25 per cent peptone	0	0	X	X0	0	0	0	0
	0.25 per cent peptone 0.5 per cent beef extract		0	X	?		0	X	?
	0.25 per cent peptone 1.0 per cent glucose	0		0	0	0		0	0
	0.25 per cent peptone 0.25 per cent ammonium sulphate		0	0	X0		0	0	X0
B. coli (A).	0.25 per cent peptone 0.5 per cent beef extract		0	X			0	X	
	0.25 per cent peptone 1.0 per cent glucose	0		0	X	0		0	X
	0.25 per cent peptone 0.25 per cent ammonium sulphate	0	0	0	0		0	X	X
B. typhi (Y. M. S.)	0.25 per cent peptone	0	0	0	0		X	0	0
	0.25 per cent peptone 0.5 per cent beef extract		0	X	X	0	0	X	X
	0.25 per cent peptone 1.0 per cent glucose	0	0	0	0	0	X	XXX	XX
	0.25 per cent peptone 0.25 per cent ammonium sulphate		0	0	0		0	0	0
B paratyphi B.	0.25 per cent peptone	0	0	0	0	0	0	0	0
B. pullorum	0.25 per cent peptone	0	0		X	0	0		X
B. aerogenes.	0.25 per cent peptone	0	0		0	0	0		0

to those already recorded have been obtained.   These will constitute part of a future publication from this laboratory.

The results given in Table IV are in marked contrast to the preceding.   The pronounced and rapid decomposition of the soluble proteins left no doubt as to the ability of gelatin-liquefying bacteria to convert them into products which no longer give the biuret reaction.   *Sp. cholerae* and *Staphylococcus aureus* were, however, much less active than the others.

It may be of interest to note the sparing action of glucose on the proteoses and peptones in the flasks which were incubated at 37°C.   At room temperature (20°C.) the protein-sparing action did not last beyond the first two weeks.

III. BACTERIAL AUTOLYSIS

The term "autolysis" has been used somewhat indiscriminately by bacteriologists.   Whether it is to denote actual decomposition of the intracellular proteins by the action of the bacteria themselves, or of certain enzymes, or whether it is meant to signify merely a liberation of intracellular substances without change in their chemical structure, is often left undetermined.   The word has for many years had definite significance, however, in biochemical literature, carrying with it the idea of self-digestion, as the term implies.   This can, of course, be its only true meaning.

It has frequently been shown that real bacterial autolysis is a common phenomenon in organisms of the *Bacillus prodigiosus* and *Bacillus pyocyaneus* type which elaborate strong proteolytic enzymes (Rettger, 1904; Levy and Phersdorff, 1902), especially under conditions of food deprivation and relatively high temperatures.   It is to be questioned, however, whether the so-called "autolysis" of cultures of *B. coli* and *B. typhi* during long incubation, and the liberation of endotoxin, as claimed by some investigators (Conradi, 1903) is a process of real self-digestion.

The present study of autolysis was carried on with certain well-known proteolytic organisms, namely *B. prodigiosus, Proteus vulgaris, Ps. fluorescens (B. fluorescens liquefaciens*, Flügge), *B. subtilis* and *B. ramosus*, and with *B. typhi* and several different strains of *B. coli* representing the gelatin-non-liquefying class

TABLE IV

Showing the behavior of certain gelatin-liquefying bacteria to Witte's peptone

ORGANISMS	MEDIA	DECOMPOSITION OF PROTEOSES AND PEPTONES							
		At 37° C.				At 20° C.			
		1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks	4 weeks
Staphylococcus aureus	0.5 per cent peptone	0	0	0	0	0	0	0	0
	0.25 per cent peptone	0	X	0	X	0	X		X
Staph. albus	0.25 per cent peptone	XXXX	XXXX		XXXX	X	XXX		XXXX
Sp. cholerae	0.25 per cent peptone	0	0		XX	0	?		XX
B. ramosus	0.25 per cent peptone	no growth				0	0		XXXX
B. cereus	0.5 per cent peptone	XXXX	XXXX		XXXX	X	XXXX	XXX	XXXX
	0.25 per cent peptone	X	Color interference			X *	XX	XXX	XXXX
Proteus vulgaris (S)	1.0 per cent glucose	0	0	0	0	0	0	0	0
	0.25 per cent peptone	0	XXXXX	XXXXX	XXXXX	0	XXXXX	XXXXX	XXXX
	0.25 per cent ammonium sulphate	0	0	0	0	0	0	0	0
Proteus vulgaris (F)	0.25 per cent peptone	0	0	0	0	0	0	XXX	XXX
	1.0 per cent glucose	0	0	0	0	0	0	0	0
	0.25 per cent peptone	XX	Color interference			XXXXX	XXXXX	XXXXX	XXXXX
	0.25 per cent ammonium sulphate	0	0	0	0	0	0	0	0
Proteus mirabilis	0.25 per cent peptone	0	0	0	0	0	0	XXX	?
	0.25 per cent peptone	0	0	0	0	0	XX	XXX	XXXX
	1.0 per cent glucose	0	0	0	0	0	0	0	0
	0.25 per cent peptone	XXXX	XXXX	XXXX	XXXX	XXX	XXX	XXX	XXXX
	0.25 per cent ammonium sulphate	0	0	0	0	0	0	0	0

	Medium									
B. subtilis	0.25 per cent peptone	XXXX	XXXX	XXX	0	XXXX	XXXX	XXX	XXXX	XXXX
	0.25 per cent peptone, 0.5 per cent beef extract	XXXX	XXXX	XXX		XXXX	XXXX	XXX	XXXX	XXXX
	0.25 per cent peptone, 1.0 per cent glucose	0	0	0	XX	0	0	0	XXX	XXXX
	0.25 per cent peptone, 0.25 per cent ammonium sulphate	XXXX	XXXX	XXX		XXXX	XXXXX	XXXX	XX	XXXX
	2.0 per cent peptone	0	XX	XX		XXXXX	XX	XXXX	XXX	0
	0.5 per cent peptone	XX	XXXXX	XXXXX	X	XXXXX	?	XXXXX	XXX	XXXX
B. prodigiosus	0.25 per cent peptone	0	0	0	X	0	0	0	0	XXXX
	1.0 per cent glucose	XX	XXXX	XXXX	X	XXXX	XXX	XXX	XXX	XXX
	0.25 per cent peptone, 0.25 per cent ammonium sulphate	0	XXXXX	XXXXX		XXXXX	XXX	XX	XX	XX

NOTE. In all of these tables 0 indicates no reduction in the amounts of proteoses and peptones, and XXXXX the complete disappearance of those proteins from the media, as indicated by the biuret test.

of bacteria. The experiments fall into two distinct groups; in the first, tests were made as to the ability of bacteria to digest or destroy their own proteins under highly favorable conditions of temperature and environment. Representative gelatin-liquefying and non-liquefying organisms were employed. The second set of experiments had as its object a study of the fate of purified egg albumin, Witte's peptone, and dialyzed proteoses when added in small amounts to autolyzed bacterial suspensions or to suspensions which had the necessary conditions for proteolysis providing the organisms were capable of digesting themselves.

The different organisms were grown on slant agar and, in a few instances, on potato. The surface growths were washed off with distilled water and transferred to sterile bottles. These suspensions which were of the consistency of a thin paste were incubated at 37°C. with 5 per cent toluol. Definite amounts of this material were tested from time to time by the quantitative biuret method, a 0.25 per cent solution of peptone being employed as a standard for color comparison. The exact plan of the experiments and the results are given in the following tables.

TABLE V

Autolysis experiments with gelatin-liquefying bacteria

ORGANISMS	BIURET REACTIONS AND COLOR COMPARISONS WITH STANDARD PEPTONE SOLUTION (0.25 PER CENT)									STANDARD PEPTONE (0.25 PER CENT)
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	15th day	
B. subtilis....... {	0.05						0.0			
	0.25									
	0.07		Faint			0.0				
										1.0
B. prodigiosus... {	5.0	4.5		1.0		0.05				
	2.0		1.0		Faint		0.0			
	2.0		1.5		1.0		0.5		Faint	
Prot. vulgaris ... {	1.0		Faint				0.0			
	1.0		Faint				0.5			
B. ramosus........	0.15	0.10	0.03							

NOTE: The above figures are based on the relative strengths of color obtained in the tests, each being compared with the degree of color given by 1.0 cc. of the standard (0.25 per cent) solution of Witte's peptone which is taken as 1.0. In all of the tests 1.0 cc. of the autolysis material was employed.

TABLE VI

Autolysis experiments with different strains of Bacillus coli

ORGANISMS	BIURET REACTIONS AND COLOR COMPARISONS WITH STANDARD PEPTONE SOLUTION (0.25 PER CENT)														STANDARD PEPTONE
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	9th day	12th day	14th day	18th day	20th day	30th day	50th day	
B. coli I......	2.0	2.0	2.0	?			1.0*								
B. coli II....	1.0		1.0	1.0				1.0	1.0						
B. coli III...	1.0		1.0			1.0	1.0	1.0	0.75†						1.0
B. coli IV....	2.5			2.5								2.5†			
B. coli V.....	1.0			1.0								1.0			
B. coli VI....	1.0	1.0	1.0									1.0			
B. coli VII...	1.0								1.0						
B. coli VIII..	0.1								0.1		0.1		0.1	0.1	

*Contamination with Subtilis-like organism.

† Developed a condition which rendered the test fluid cloudy, and hence made quantitative study impossible.

TABLE VII

Combined autolysis experiments with gelatin-liquefying and non-liquefying bacteria

ORGANISMS	BIURET REACTIONS AND COLOR COMPARISONS WITH STANDARD PEPTONE SOLUTION (0.25 PER CENT)														STANDARD PEPTONE
	1st day	2nd day	3rd day	4th day	6th day	7th day	8th day	10th day	25th day	30th day	40th day	75th day	110th day	120th day	
B. subtilis I.....	1.0		0.0												
B. subtilis II....	0.2		0.0												
B. subtilis......	0.2					0.0									1.0
Ps. fluorescens..	0.5			0.3				0.1		0.0					
B. coli (H)......	0.4	0.4		0.4			0.4		0.4	0.4	0.4	0.4	0.4	0.4	
B. immobile (fluorescens non-liquefaciens).........	0.6			0.6				0.6		0.6					
B. typhi (Ho)...	0.8		0.8		0.8			0.8*	0.0*						
M. luteus (cereus flavus)...	0.5		0.5			0.5			0.5						

* At the end of ten days of incubation of the autolysis bottle (B. typhi) 2 cc. of completely autolyzed B. subtilis material was added. After eight days the contents of the bottle failed to give a biuret reaction.

In the following experiments 1 cubic centimeter of autolyzed material or of bacterial suspensions which had been incubated long enough to allow of self-digestion, was added to 5 cc. of purified egg albumin, to Witte's peptone and to purified proteose. Biuret tests were made after definite periods of incubation at 37°C. In each experiment the original biuret reaction is the same for all of the flasks including the control.

TABLE VIII

Proteolytic action of autolysis material on purified egg albumin. 1.0 cc. of suspension added to 5.0 cc. of the albumin solution

ORGANISMS	BIURET REACTIONS AND COLOR COMPARISONS WITH EGG ALBUMIN CONTROL				
	1st day	3rd day	5th day	12th day	20th day
B. prodigiosus..................	1.0		0.5	Faint	0.0
B. coli I.......................	1.0	1.0	1.0	1.0	1.0
Control......................	1.0	1.0	1.0	1.0	1.0

TABLE IX

Action of autolysis material (1.0 cc.) on solution of purified proteoses (5 cc.)

ORGANISMS	BIURET REACTIONS AND COLOR COMPARISON WITH PROTEOSE SOLUTION CONTROL				
	1st day	2nd day	3rd day	6th day	10th day
B. prodigiosus I...............	1.0		Faint		
B. prodigiosus II..............	1.0		Faint		0.0
Prot. vulgaris I...............	1.0	0.5	?	Faint	
Prot. vulgaris II..............	1.0			0.5	
B. coli I......................	1.0	1.0	1.0	1.0	1.0
B. coli II.....................	1.0	1.0	1.0	1.0	1.0
B. coli III....................	1.0	1.0	1.0	1.0	1.0
B. coli IV.....................	1.0	1.0	1.0	1.0	1.0
Control......................	1.0	1.0	1.0	1.0	1.0

TABLE X

Action of autolysis material (1.0 cc.) on solution of Witte's peptone (5 cc. of a 5 per cent solution). Gelatin liquefiers

ORGANISMS	BIURET REACTIONS AND COLOR COMPARISONS WITH PEPTONE CONTROL					
	1st day	2nd day	4th day	6th day	12th day	20th day
B. prodigiosus.......	5.0	4.0	2.0			
B. prodigiosus II....	5.0	4.0	2.5	1.0	0.5	Faint
Prot. vulgaris........	5.0	4.0	3.0	2.0	1.5	1.0
Control...........	5.0		5.0		5.0	5.0

NOTE: The controls were prepared by adding 1 cc. of distilled water to 4 cc. of the given peptone solutions.

TABLE XI

Action of autolysis material (1 cc.) on solution of Witte's peptone (5 cc. of a 0.25 per cent solution). B. subtilis and B. coli comparison

ORGANISMS	BIURET REACTION AND COLOR COMPARISONS WITH PEPTONE CONTROL				
	1st day	2nd day	4th day	8th day	12th day
B. subtilis I....................	0.25	0.13	0.08	0.0	
B. subtilis II.................	0.25	0.20		0.12	
B. coli I......................	0.25	0.25		0.25	0.25
Control....................	0.25	0.25		0.25	0.25

NOTE: This experiment was repeated with larger amounts of peptone.   The results were practically the same as in this table.

The above tables show clearly the ability of bacteria to digest themselves.   This property appears to be confined, however, to organisms which are known to elaborate a proteolytic enzyme—the gelatin-liquefying group.   Not only do the organisms of this group rapidly destroy their own protein under favorable conditions of autolysis, but they readily attack and decompose egg albumin, peptones and partially purified proteoses when the autolyzing materials are brought in contact with these foreign proteins.

On the other hand, the gelatin-non-liquefying organisms employed in these experiments were unable to effect any change in the protein content of the respective suspensions, at least in so far as may be judged by the biuret tests.   Furthermore, other

proteins, when added to the bacterial suspensions after periods of preliminary incubation, remained unaffected. In every instance where the test was satisfactorily carried out the quality and degree of color obtained in the biuret test remained unchanged, as is readily seen by comparisons with the controls or with the standard peptone solution.

Autolysis of the bacterial cells was always accompanied by a change in the staining properties of the individual organisms. In many cases, as for example in the complete autolysis of *B. subtilis* material, the bacilli took on only a faint color; and the presence of numerous fine granules presented a picture far from the normal. A difference in staining properties was also occasionally observed in the organisms of the *B. coli* type, but this was never marked, and was not due to actual destruction of the cell protein, as was shown always by the biuret test. The change was due to some process other than autolysis, as for instance "washing" or "laking" of the bacterial cells.

GENERAL DISCUSSION AND CONCLUSIONS

The results of the present investigation strongly indicate that bacteria are unable to attack and bring about the decomposition of proteins without the aid of enzymes or other proteolytic agents. This applies not only to the more complex proteins like egg albumin, but in all probability to albumoses and peptones as well. Coagulated albumin shows the same resistance to the direct action of bacteria of both the gelatin-liquefying and non-liquefying types as the unheated and unchanged native proteins.

By means of the quantitative biuret test of Vernon the disappearance of proteoses and peptones from solutions serving as test or culture media may be readily demonstrated. This method has been of much value to us in the present investigation. It is being employed for the determination of other proteins also, as for instance casein in the form of nutrose.

Even during prolonged incubation of flasks containing the necessary inorganic salts for bacterial metabolism, together with proteoses or Witte's peptone, little if any loss of these soluble

proteins could be observed if the flasks had been inoculated with members of the colon-typhoid group or with other gelatin-non-liquefying bacteria. On the other hand, organisms which are known to elaborate proteolytic enzymes, as for example *B. prodigiosus* and *B. subtilis*, rapidly brought about destruction of the proteins. Test media containing purified coagulated egg albumin or dialyzed proteoses as the only possible source of available nitrogen were, with few exceptions, not attacked, how-ever, even by the gelatin-liquefyers, if the inoculations were made with but comparatively few organisms and from a culture less than twenty-four hours old.

The slight reduction in the amount of "peptone" which was observed in a few instances may have been due to agents other than enzymes or bacterial cells, as for instance acids and am-monia. It is significant that such reductions did not become apparent until at least two to three weeks after the time of inoculation. These slight losses in the soluble proteins, if they were losses, usually occurred in flasks containing luxurious growths, and may possibly be due to adsorption by the bacteria and other suspended matter and by the walls of the flasks which were more or less coated. The possibility of the occurrence of small amounts of a proteolytic enzyme having the properties of erepsin (Cohnheim, 1901, Vernon, 1904) cannot be ignored. However, if such an enzyme is produced by organisms of the *B. coli* and *B. typhi* type it is of little importance, as no indi-cations of any proteolytic action whatever were apparent during the first two weeks, and since only very minute quantities can be produced even under the most favorable cultural conditions.

The statement that purified albumin and dialyzed proteoses were not attacked even by gelatin-liquefying bacteria if the test fluids were inoculated with few organisms taken from very young cultures may appear at first paradoxical. The results, which are in harmony with those of Bainbridge and the earlier investi-gations in this laboratory on purified albumins, readily admit of an explanation. When the test medium contains no other possi-ble source of nitrogen for cell metabolism besides the purified protein it is not attacked by any bacteria unless a sufficient

amount of the inoculating material is introduced to carry with it the necessary enzyme to bring about cleavage of the protein. In a medium containing nitrogen which is directly available, bacterial multiplication will take place, though the number of bacteria introduced is small. If such a medium also contains protein, and if the organism is one which under favorable conditions elaborates a proteolytic enzyme, the protein undergoes cleavage as the result of the enzyme action. These points have been demonstrated repeatedly.

What are some of the important sources of nitrogen available for bacterial metabolism without the aid of an enzyme? Our attention will naturally be directed to amino acids which in animal physiology are now known to play such an important part in nutrition. Witte's peptone contains amino acids which may be demonstrated readily by any of the well-known tests, particularly the Sörensen method (Sörensen, 1908). The amount of amino acids present in the American brands of peptone is considerably greater than in the Witte product. This undoubtedly explains why we have consistently obtained more luxuriant, though not necessarily more characteristic, bacterial growths in media which contained the American products than in the standard Witte.

It appears at this time indeed probable that so-called "peptone media" largely owe their value as culture media to the amino acids and perhaps other nitrogenous substances which readily give up their nitrogen as the result of direct bacterial action, and unless bacteria are present which elaborate proteolytic enzymes, little if any of the proteoses and peptones in the medium is utilized. Indeed it may be necessary for us to go even further than this, and to adopt the view that the bacterial cell can not utilize any protein until after it has been broken up by some other agent and the nitrogenous portion converted into simple form. If this view should obtain it will be necessary for us to alter materially our conception of the value of peptone, nutrose, and other soluble as well as insoluble proteins as culture media, especially in so far as the group of gelatin-non-liquefying bacteria is concerned.

Such a view as is tentatively presented here is certainly in harmony with the results of Loewi (1902) Abderhalden and others who in elaborate investigations have shown that animals like the dog may be maintained in nitrogenous equilibrium for long periods of time when fed on a diet in which all protein material had been replaced by the products of prolonged digestion of proteins. These experiments imply that the cleavage products of the proteins are resynthesized in the animal body.   According to Abderhalden no cells can directly assimilate and utilize foreign food material.   Complex nitrogenous food material must be prepared for the cell through enzyme action.   This breaking down and the reconstruction of food are just as necessary as it is to reduce a church to the very bricks which constitute it before it can be converted into a school-house.   This may perhaps apply to the bacterial cell as well as in the field of animal cell nutrition.

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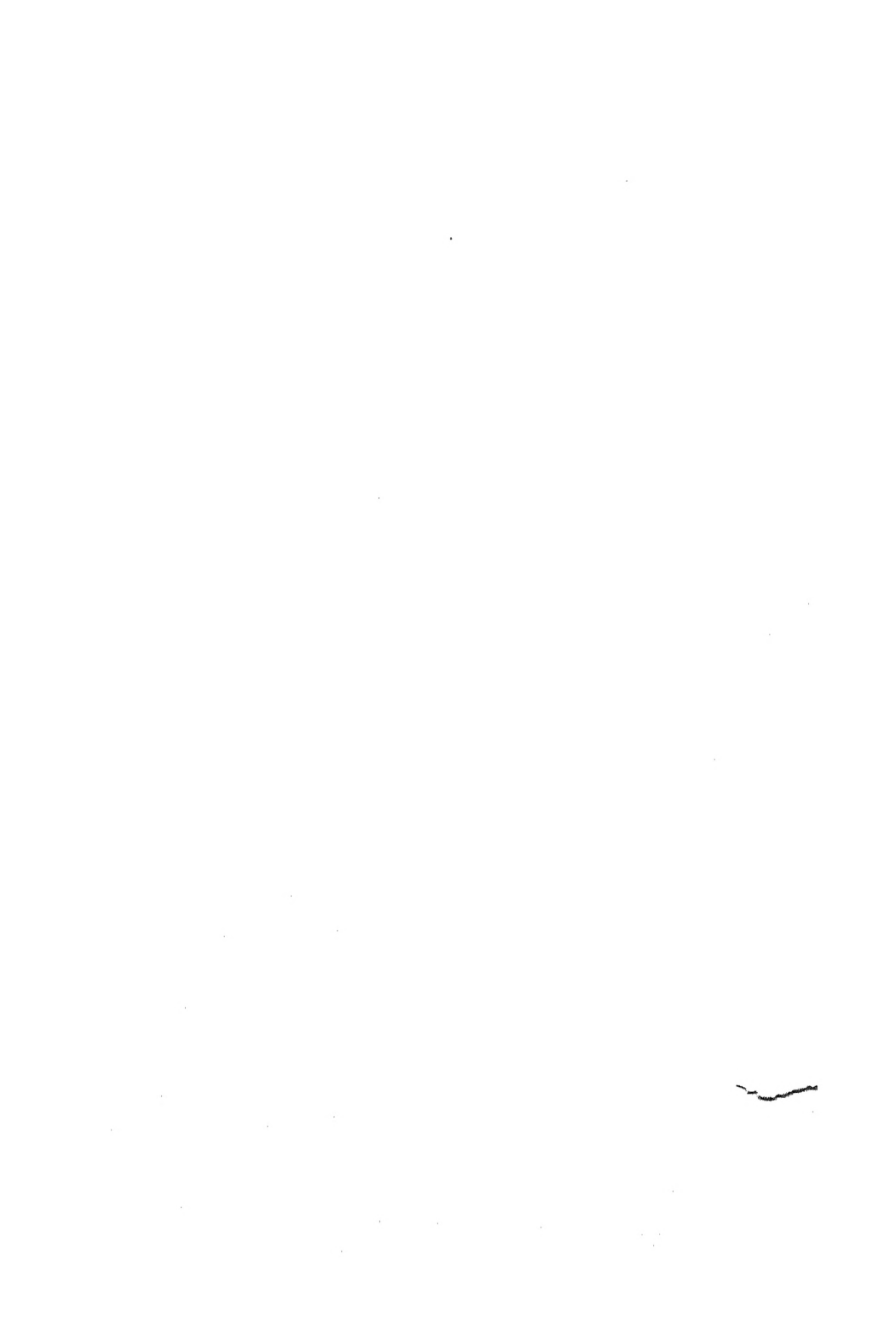
STUDIES ON SOIL PROTOZOA AND THEIR RELATION TO THE BACTERIAL FLORA. I[1]

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I. INTRODUCTION

The occurrence of protozoa in soil

The knowledge that protozoa occur in soil dates back nearly
as far as does the science of microbiology, but it is only recently
that specific studies have been directed at the micro-fauna of
the soil.   Müller (1887) reported studies concerning some soil
protozoa which he thought played a part in the destruction
of organic tissue, and thus were to be considered as important
agents in the formation of humus.   Celli and Fiocca (1894)
studied the protozoa of the soil and described several forms of
amoebae obtained from this source.   Beijerinck (1896) described
an amoeba which occurred in his cultures of nitrifying bacteria,
and later (1901) called attention to a variety of amoebae, monads
and infusoria which appeared in cultures with Azotobacter.
Frosch (1909) isolated a number of saprophytic amoebae from
garden soil similar to those found in the intestinal tracts of ani-
mals.   Tsujitani (1908), likewise described some amoebae which
occur in soil.   Hiltner (1907) noted many types of protozoa
which appeared in cultures made from soil, and stated that
these organisms certainly do not play an unimportant rôle.   He
noted the presence of various ciliates, flagellates, and amoebae,
some of which he said were often present in unusually large
numbers.   Störmer (1907) also studied the protozoan fauna, and
demonstrated that the soil contains a considerable number of
these organisms, especially amoebae.

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Within the past few years more attention has been directed toward the soil protozoa with the result that they have been demonstrated to be of general occurrence in the soils of those parts of the world which have been studied.   In England, Russell and Hutchinson (1909) (1913), Russell and Golding (1912), Goodey (1911), Martin (1912), and Martin and Lewin (1914) have noted the constant presence of protozoa in soil.   Similar observations have been made by Wolff (1909) (1912), Francé (1911), Killer (1913), Emmerrich, Leiningen and Loew (1912), and Cunningham and Löhnis (1914) in Germany; by Lodge and Smith (1912), Gainey (1912), Rahn (1913) and Sherman (1914) in the United States; by Cauda and Sangiori (1914) in Italy, Peck (1910) in Hawaii, and Greig-Smith (1912) in Australia; while Loew (1911) (1913) has observed them in the Alps, in Porto Rico, the Island of Borkum, and in Japan.   Important contributions to our knowledge of the types of protozoa which occur in soil have been made by Wolff (1909), Francé (1911), Goodey (1911) (1914), Martin (1912), and Martin and Lewin (1914).

The relation of protozoa to bacteria

It is well known that bacteria constitute the chief food for many types of protozoa.   Many of the ciliates in particular are especially destructive to bacteria, although this property is possessed by the other classes as well.   Indeed, Calkins (1901) has said that probably all protozoa ingest bacteria with the exception of the parasitic forms and those which live on other protozoa. This view, however, has been modified considerably in recent years and it is now known, as is stated by Minchin (1912), that a number of protozoa are saprozoic in nature and obtain their food by absorption.   A considerable portion of the non-parasitic flagellates belong to this class.

In many places in nature bacterial development is limited by the action of predatory protozoa.   Huntemüller (1905) and Korshun (1907) have proven that the micro-fauna plays an important part in the purification of water.   The possibility that protozoa are inimical to the soil bacteria has only very

recently received serious consideration, probably because of the lack of evidence that these organisms exist in an active, free-living condition in the soil.

The phagocytic theory of soil fertility

Interest in the soil protozoa was given a great stimulus in 1909 when Russell and Hutchinson (1909) of the Rothamsted Experimental Station announced their theory which involves the protozoa as a factor detrimental to the soil bacteria, and, therefore, to soil fertility. This theory, commonly known as the phagocytic theory of soil fertility, was proposed in an effort to explain the phenomena associated with the partial sterilization of soil with heat or with volatile antiseptics. The increased yields of crops obtained after partial sterilization is explained, by the sponsers of this theory, on the view that the soil protozoa prey upon the bacteria and thus act as a limiting factor on the microflora of the soil. The process of partial sterilization is thought to destroy the protozoa while the bacteria are greatly reduced, but not exterminated. When the protozoa are suppressed, the bacteria which remain are allowed to multiply unhindered and so attain numbers greatly in excess of those found in normal soils. A greater number of bacteria results in the elaboration of a greater amount of plant food, hence larger crops are produced.

No direct proof has been produced in support of this theory, but Russell and Hutchinson and their associates (1909), (1912), (1913) have presented much evidence of an indirect nature which indicates strongly that some biological factor, detrimental to bacteria, does exist in the soil.

Other views on the partial sterilization of soil

In opposition to the protozoan theory of Russell and Hutchinson, several other explanations have been advanced to account for the beneficial effects of volatile antiseptics upon the higher and lower forms of plant life in the soil. Koch (1899) claims that the antiseptic acts as a stimulant directly upon the bac-

terial flora of the soil, and likewise upon the higher plants. In support of this stimulation theory some very convincing data have been furnished by Koch (1899) (1911), Nobbe and Richter (1904), Egorov (1908), Fred (1911), Gainey (1912) and others.

The selective theory of Hiltner and Störmer (1908) holds that volatile antiseptics exert a selective action on the bacterial flora of the soil. It is thought that the soil is so changed that the subsequent development of the beneficial types of bacteria is enhanced, while the harmful forms are suppressed. These investigators believed the increased crop yields obtained to be due to the increase in the amount of plant food elaborated by the beneficial bacteria.

Bolley (1910) (1913 a) 1913 b) claims that the improvement of soil by partial sterilization is in many cases due to the destruction of certain parasite fungi which attack the plants and thus hinder their growth and development. Another function performed by volatile antiseptics according to Grieg-Smith (1911) is the liberation of plant and bacterial food through the solution of the "agricere" or soil wax.

Other points have been noted in the works of various investigators which partially account for the action of certain antiseptics in soil exclusive of their effect upon the protozoan fauna. Buddin (1914) has shown that the treatment of soil with sulphur dioxide increases the number of bacteria very appreciably without exterminating the protozoa, while certain other compounds such as pyridine cause an increase in the number of bacteria due to the fact that their decomposition products furnish an excellent source of food for the soil micro-organisms. Hutchinson and MacLennan (1914) have shown that the partial sterilization of soil with caustic lime leads to a chemical breaking down of some of the organic matter of the soil and thus stimulates the subsequent activities of the bacteria. Fred (1915) in his work on the action of carbon bisulphide in soil has demonstrated that all of this compound does not evaporate when added to the soil, but that some of it remains and is changed to sulphates.

Some workers believe that the value of partial sterilization by heat is due to the destruction of soil toxins which limit the

activities of the micro-organisms and plants. Whether toxins occur in soil is a disputed question, but Whitney and Cameron (1904) (1910), Schreiner and Reed (1907 a) (1907 b), and Pickering (1910 b) have demonstrated quite satisfactorily that plant toxins do exist; while Bottomley (1911) and Greig-Smith (1911) have submitted data which point to the existence of bacterio-toxins in the soil.

Others explain the beneficial action of heat by the changes it produces in the soil compounds. Frank (1888), Pickering (1910 a), Lyon and Bizzell (1910), Stone (1912) and others have demonstrated an increase in the amount of soluble plant and bacterial food in partially sterilized soil. Pfeffer and Franke (1896) and Krüger and Schneidewind (1899) have shown an increased assimilation by plants in heated soils. Pickering (1910 b), Wilson (1914) and others have further proven that heat may also produce a toxic compound in the soil, the toxicity increasing with an increase in the temperature used, so that soils heated to very high temperatures have a detrimental effect upon plant growth.

The effect of protozoa on the bacterial flora of the soil

There has been only a limited amount of work done upon the action of the protozoa in soil aside from the indirect evidence which has been acquired in the study of partial sterilization. Russell and Hutchinson (1913) have failed in their attempts to reduce the number of bacteria in partially sterilized soil by the addition of mass cultures of protozoa; Lipman, Blair, Owen and McLean (1910) in their work on ammonification in soil were unable to detect any influence upon this process due to the protozoa; while Grieg-Smith (1912) obtained entirely negative results in his efforts to show that protozoa are detrimental to the bacterial flora of the soil. Cunningham (1914), on the other hand, claims to have demonstrated that protozoa do limit the number of bacteria in soil.

Outline of work undertaken

Preliminary to any work on the part played by the soil protozoa, two essential points should be established: (1) whether protozoa occur in soil in numbers sufficient to be a factor in soil fertility; and (2) whether protozoa lead a trophic life in the soil. Unless these two points can be settled in the affirmative, it would appear that discussions concerning the rôle played by the micro-fauna of the soil must be considered more or less futile. The first part of this work was therefore directed toward getting more definite information as to the number of protozoa in soil and the nature of their existence therein.

A study was made of the effect of protozoa upon the bacterial flora of the soil by the isolation of animal-pure cultures of some representative soil protozoa and inoculation into protozoa-free cultures of soil bacteria, in solutions and in soil, and also by the comparison of the activities of bacteria in sterilized soil reinoculated with normal soil and with "protozoa-free soil."

The last part of the work was devoted to a study of the action of volatile antiseptics in soil in an effort to throw some light on the part played by the protozoa.

II. THE NUMBER OF PROTOZOA IN SOIL

Present status

Although the soil protozoa have attracted considerable attention in recent years, few data are at hand showing the number of such organisms actually found in normal soils. Störmer (1907) showed that fertile soils sometimes contain several thousand amoebae per gram as determined by the agar plate method of enumeration. Hiltner (1907) reported the finding of large numbers of protozoa in soil, and said that flagellates and amoebae had been found in numbers reaching millions per gram. He did not report the specific data upon which this statement was based, nor did he give the details of the method by which the numbers of protozoa were determined. Lodge and Smith (1912), on the

other hand, investigated field soils of Massachusetts and claimed that the number of protozoa present would have to be increased many fold in order to be considered a factor in the limitation of bacterial numbers. Gainey (1912) studied the protozoa in Missouri soils and likewise concluded that the number was not sufficient to be a factor in soil fertility. Rahn (1913) by use of the dilution method determined the number of protozoa in Michigan soils. From the limited data submitted by him it would appear that the soil contains about one thousand protozoa per gram. Killer (1913) tested various methods for the determination of the soil protozoa but concluded that all of the methods now known are of limited and doubtful value. Recently Cunningham (1914) has reported studies of German soils which he has demonstrated to contain quite large numbers of protozoa.

Methods

The dilution method, which has been employed to some extent for estimating the number of protozoa in soil, has been used in this work. It is obviously impossible to devise one medium which will favor equally the development of all of the various forms of unicellular animal organisms found in the soil. In the preliminary work undertaken, various media were used with the idea of finding which forms of protozoa are most abundant in soil, and which media are best adapted to those particular forms.

From observations on several media it was early observed that the flagellates make up the greater portion of the protozoan population of the soils which were studied. The survey of media has not been very extensive, but a dilute soil extract has given the most satisfactory results for determining the number of flagellates. This medium is also well suited for the growth of ciliates and amoebae. The use of soil extract seems appropriate as it would appear that the organisms favored by this medium would be the ones most likely to be leading a trophic life in the soil. One per cent hay extract, which has been used by most investigators in the study of the soil protozoa, has not given as

satisfactory results as has soil extract, in the comparison made at this laboratory.

Soil extract prepared by boiling one part of soil in three parts of distilled water, filtering clear, and adding a small excess of $CaCO_3$, has been used. This has been modified by using only one part of soil in nine parts of water and one part of a one per cent hay extract, plus $CaCO_3$. The presence of this small amount of hay extract does not appear to exert any inhibitory effect upon the flagellates, while the ciliates seem to be benefited. In sampling soil not less than ten grams have been taken, and duplicate dilutions have always been made. The cultures have been incubated at 20°–25°C. and examined every few days for a period of about ten days.

Results

The data obtained on twelve Wisconsin soils, representing various types under different treatments, are tabulated in Table I.

TABLE I

Approximate number of protozoa in various Wisconsin soils

NO.	TYPES OF SOIL	TREATMENT OF SOIL	DILUTIONS			
			1/1,000 gram		1/10,000 gram	
			A	B	A	B
1	Clay loam	Apple orchard	+	+	+	+
2	Clay loam	Blackberry patch	+	+	−	+
3	Rich loam	Garden	+	+	+	+
4	Sandy loam	Tobacco field	+	+	+	−
5	Peat soil	Uncultivated	+†	+	+	−
6	Loam soil	Pasture	+	+	−	−
7	Clay loam	Vineyard	+*	+*	−	+
8	Loam soil	Alfalfa field	+	+	−	+
9	Clay loam	Not planted	+*	+*	+	−
10	Sandy loam	Clover	+	+	−	−
11	Sandy loam	Castor bean bed	+	+	−	+
12	Sandy loam	Corn field	+	+	+	+

A and B represent the duplicate samples of each soil.

+ = presence of protozoa.

− = no protozoa present.

* Colpoda found in addition to flagellates.

† Amoebae found in addition to flagellates.

That the high protozoan content noted above is not peculiar to Wisconsin soils is shown by the data obtained from three Virginia soils and one Tennessee soil given in Table II. These samples were from representative soils in a good state of cultivation.

The figures of the foregoing tables show that, in the sixteen soils recorded, in every soil protozoa were found in both samples of the 1/1,000 of a gram dilution, while of the total of 32 dilutions representing 1/10,000 of a gram each, 18 revealed the presence of protozoa. When we consider that, (1) it is extremely improbable that every individual protozoan grows when taken

TABLE II

The approximate number of protozoa in Virginia and Tennessee soils

NO.	SOURCE	TYPE OF SOIL	TREATMENT OF SOIL	DILUTIONS			
				1/1,000 gram		1/10,000 gram	
				A	B	A	B
1	Virginia	Clay	Garden	+	+	+	+
2	Virginia	Clay loam	Wheat field	+	+	−	+
3	Virginia	Loam	Grass field	+	+*	+	+
4	Tennessee	Compact red clay	Corn field	+	+	−	−

A and B represent the duplicate samples of each soil.

+ = presence of protozoa.

− = no protozoa present.

* *Balantiophorus elongatus* found in addition to flagellates.

from the soil and introduced into a liquid medium; (2) the liquid medium used is probably not adapted for the growth of all protozoan inhabitants of the soil; and (3) some of the samples representing only 1/10,000 of a gram contained three distinct types of animal organisms, the statement that the average fertile soil contains about 10,000 protozoa per gram seems conservative.[2]

In cultures inoculated with 1/1,000 of a gram of soil, a variety of flagellates are usually to be observed. As seen from the above

[2] It should be borne in mind that these data are not intended to give an accurate estimate of the numbers of protozoa in soil, but only to show whether they occur in numbers sufficient to be regarded as a possible factor in soil fertility.

data, the ciliates do not ordinarily occur in numbers approximating 1,000 per gram. *Colpoda cucullus* which has been widely noted as an inhabitant of soil, appears to be the most generally distributed ciliate. This organism may be very often, but not constantly, found in 1/100 of a gram of soil. In one Virginia soil (Table II), *Balantiophorus elongatus* was found in the 1/1,000 of a gram dilution. With the methods employed, it would not be safe to draw any conclusions regarding the amoebae other than that they do not occur in numbers nearly as great as do the flagellates in ordinary soils. Although the culture media and period of observation used are not adequate for work with the amoebae, it is worthy of note that in the peat soil, containing a high humus and water content (in which we should expect to find numerous amoebae) the method was of sufficient service to show their presence in 1/1,000 of a gram in one of the two samples taken. In spite of the limitations of the methods used, amoebae would probably have been revealed had they been of general occurrence in large numbers. Using the same medium and inoculating with a large amount of soil, amoebae have been observed as early as the third day.

In the dilutions of 1/10,000 of a gram, with one exception, only four general types of protozoa have as yet been noted: *Monas sp. Dimorpha radiata* (?) and two other flagellates which have not been identified.

III. THE GROWTH OF PROTOZOA IN SOIL

Previous work

In order to correlate the protozoa with any of the vital functions of the soil, it is first necessary to demonstrate that they are active in soils of normal moisture content. Goodey (1911) made a very careful study of the ciliates of the soil and concluded that these organisms exist in soils of normal moisture content only in the encysted condition. When free water was to be found on the soil active protozoa could be demonstrated, but no active ciliates could be detected in ordinary soils. Russell and Golding (1912) working with water-logged, "sewage sick" soil, demon-

strated the presence of active protozoa. Under these conditions, as was pointed out by Goodey, many protozoa may become active. When we consider the minute size of these organisms it would appear obvious that they may become active whenever the soil is in a saturated condition. Martin (1913) by means of a special method which he has developed has been able to prove definitely that soil contains active protozoa. Cunningham (1914) has collected data which indicate that some of the protozoa exist in soil in the active state.

Experimental

In order to see if any of the soil protozoa are active, the following experiment was performed. Four pots each containing two kilograms of sterilized soil with an optimum moisture content of about 17 per cent were inoculated with two grams each of a normal soil, which showed 10,000 protozoa per gram by the dilution method. These pots were then brought up to varying moisture contents by the addition of sterile water, as follows:

	per cent H_2O
Pot No. 1	11
Pot No. 2	12
Pot No. 3	15
Pot No. 4	22

Since one gram of normal soil, with a protozoal count of 10,000 per gram, was used to inoculate 1,000 grams of sterile soil, the resulting mixture should contain, approximately, only 10 of these organisms per gram. After fifteen days incubation at 20 to 25°C., these pots were sampled, and in every case flagellates were found in the 1/10,000 dilutions. This could not be explained except on the assumption that these animal organisms had undergone rapid multiplication. The forms found in greatest numbers in these pots were the same forms which were noted as the predominating types in normal soils. *Monas sp.* did not occur in the 1/10,000 dilution cultures from the soils containing only 11 and 12 per cent moisture. In the case of pot 4, with a moisture content of 22 per cent, *Colpoda cucullus* was found in the

1/100 dilution, showing that this organism was probably active in the soil containing this high amount of water.

To obtain more definite data on this point, with known organisms, an experiment was performed in which pots of sterilized soil were inoculated with animal-pure cultures of three of the types of flagellates mentioned in the first part of this paper. The pots were inoculated with 0.1 gm. each of soil cultures of the respective organisms per 1,000 grams of sterile soil. The water content was then held at 15 per cent (a little below normal for a soil of this type). Determinations for protozoa were made immediately after inoculation, and then at intervals for fifteen days. The results obtained are given in Table III.

TABLE III

The multiplication of three types of protozoa in soil. (H_2O *content 15 per cent.*)

ORGANISM	NUMBER PER GRAM				
	Start	6 days	9 days	12 days	15 days
Monas (sp)...................	1	10	10	1,000	10,000
D. radiata....................	1	100	100	1,000	100,000
Flagellate A.................	10	100	1,000	10,000	100,000

In similar tests using *Colpoda cucullus, Balantiophorus elongatus* and *Oxytricha sp.* no multiplication could be detected during a period of thirty days. These results appear to substantiate the data of Goodey on the ciliates.

A point which probably deserves mention in this connection, is that when sterilized soil is inoculated with normal soil, the protozoan fauna rises in numbers above that of normal soil, just as does the bacterial flora. In other words, it is probable that the micro-organic balance remains about the same. In Table IV are given the results obtained on five pots of sterilized soil which were inoculated with normal soil.

Higher dilutions have not been tried, but it is not unlikely that under such conditions the number of protozoa may reach 1,000,000 per gram. This fact is not of great significance, but it has a practical application in indicating that the subsequent work on the relation of the soil protozoa to the bacterial flora

may be carried out in a sterilized soil medium without causing an apparent disturbance in the balance between these two classes of organisms.

TABLE IV

Approximate number of protozoa in reinoculated sterile soil

POT NO.	DILUTIONS			
	1/10,000 gram		1/100,000 gram	
	A	B	A	B
1	+	+	+	+
2	+	+	+	—
3	+	+	+	+
4	+	+	—	+
5	+	+	+	+

A and B represent the duplicate samples of each pot.

+ = presence of protozoa.

— no protozoa present.

IV. THE BEHAVIOR OF BACTERIA IN SOILS CONTAINING PROTOZOA AND FREE FROM PROTOZOA

Methods

In an effort to show the influence of the soil protozoa upon the bacterial flora the following method was employed. Pots of soil covered with a layer of non-absorbent cotton between layers of cheese cloth, to prevent reinfection, were sterilized in the autoclave under fifteen pounds steam pressure for one hour. Some of the pots were then inoculated with unsterilized soil in order to introduce all of the biological factors peculiar to normal soils, while the others were inoculated with a special soil containing a varied mixture of soil bacteria but free of protozoa.

This "protozoa-free soil" was made up of a mixed flora obtained from several different soils by the isolation of as many kinds of bacteria as could be obtained. For this purpose several different soils were plated out on beef extract, casein, Heyden and Ashby's agars and all of the colonies which developed transferred to sterile soil. Other portions of soil which had been partially sterilized by heat sufficiently to kill all protozoa

were also added to this soil culture. The flora was made more complex by the addition of dilutions of various soils which represented 1/1,000,000 of a gram, and which contained no protozoa. It would seem that the addition of these dilutions would seed the soil culture with those types of bacteria which predominate in normal soils. The protozoa-free soil so prepared was protected from contamination and its freedom from protozoa was verified at frequent intervals.

In all of the experiments in this part of the work at least one kilogram of soil was used in each pot. For most of the trials larger amounts were used according to the size of the pots. The moisture content of the soils was maintained at about two-thirds of their water holding capacities.

For the determination of the number of bacteria the soils were plated on Heyden agar and the counts made after ten days incubation at laboratory temperature.

The number of bacteria in soils containing protozoa and free of protozoa

Many determinations have been made of the number of bacteria in sterilized soils which were reinoculated with normal soil and with the special protozoa-free soil previously described. Before the results from these tests are discussed, it should be recognized that the method is open to severe criticism. A comparison is made of two soils containing quite different flora. It is not to be expected that the flora of the artificial soil used approaches in complexity the bacterial flora of the normal soil. The number of bacteria found in these soils would probably be different even though neither be influenced by any detrimental factor. It is logical to believe that the greater number of bacteria would be found in the soil containing the more complex flora, since it would seem, the greater the variety of bacteria the greater would be the efficiency of the flora in the utilization and destruction of its own by-products.

If this view is correct, the greater number of bacteria should be found in the soil inoculated with normal soil unless the micro-

flora of this soil is limited by some harmful factor. In all of the tests which have been made *the soils free of protozoa contained greater numbers of bacteria than the corresponding soils which were inoculated with normal soil.* The results obtained in a few representative trials are given in Table V.

TABLE V

The number of bacteria in soils free of protozoa and containing protozoa
Test 1*—Incubation period two months

POT	INOCULUM PROTOZOA FREE		INOCULUM NORMAL SOIL	
	Bacteria per gram	Average	Bacteria per gram	Average
1	390,000,000		206,000,000	
2	290,000,000		110,000,000	
3	274,000,000	294,800,000	232,000,000	175,600,000
4	250,000,000		164,000,000	
5	270,000,000		166,000,000	
	Test 2—Incubation period one month			
1	340,000,000	307,500,000	106,000,000	110,000,000
2	275,000,000		114,000,000	
	Test 3—Incubation period three months			
1	230,000,000	205,000,000	142,000,000	141,000,000
2	180,000,000		140,000,000	

* Each of these tests represents a distinct experiment and not a re-count on the same soil.

An experiment was also performed to see if the above phenomena would occur on different types of soil. For this purpose a rich muck soil, a clay loam and a poor sandy soil were used. The results obtained (Table VI) showed the characteristic difference in each case.

The results obtained in the foregoing experiments can not be considered as proof that the soil protozoa are inimical to the bacteria because of the differences in the two soils under consideration. However, these data, together with the previous observations that the soil contains an adequate supply of protozoa and that some of these organisms are active, certainly appear to add weight to the theory of Russell and Hutchinson that protozoa serve as a limiting factor upon the bacteria in the soil.

The number of bacteria in different types of soil containing protozoa and free of protozoa

After fifteen days incubation

INOCULUM	NUMBER OF BACTERIA PER GRAM		
	Muck soil	Loam soil	Sandy soil
Protozoa-free..................	1,030,000,000	617,000,000	24,200,000
Normal soil....................	307,000,000	214,000,000	11,900,000

After sixty days incubation

Protozoa-free..................	210,000,000	157,000,000	21,800,000
Normal soil....................	108,000,000	70,000,000	6,700,000

The effect of the complexity of the bacterial flora upon the apparent number of bacteria in soil

As was previously pointed out the weakness in the tests in which the number of bacteria in soils containing protozoa and free of protozoa are compared lies in the fact that the flora of the two soils are different. Whether the complexity of the bacterial flora affects the apparent number of bacteria, as revealed by the agar plate count, is very important in this connection. An experiment was conducted in order to test this point.

Twelve pots of sterilized soil were divided into four groups of three pots each. The soils in group A were then inoculated with all of the bacteria which developed on six Heyden agar plates from two different soils. Group B was inoculated with A plus a mixture of all the bacteria that developed on beef extract, nutrose, and casein agar plates taken from several different types of soil and from different depths of soil. Group C was inoculated with A and B plus the "protozoa-free soil" used in the previous experiments. The pots in group D were inoculated with normal soil. After incubation periods of fifteen and thirty days at laboratory temperature, samples were taken and bacterial counts made using Heyden agar. The results obtained are given in Table VII.

The results in Table VII show very clearly that the bacteria

in soil, as determined by the plate culture method, are diminished
with an increase in the complexity of the flora.   The soils used
were all free of protozoa with the exception of those in Group D,
yet there is a continual decrease in the number of bacteria found
in each group as the number of kinds of bacteria is increased.   It
will be noted that there was a greater difference obtained in the
bacterial counts between Groups B and C, neither of which
contained protozoa, than there was between C and D, one of
which contained protozoa while the other was free of these organ-
isms.   It is very probable that this decrease in the number of

TABLE VII

Effect of complexity of flora upon the apparent number of bacteria in soil

Fifteen days

NO.	NUMBER PER GRAM			
	Group A	Group B	Group C	Group D
1	500,000,000	460,000,000	220,000,000	190,000,000
2	600,000,000	420,000,000	240,000,000	250,000,000
3	580,000,000	460,000,000	270,000,000	270,000,000
Average........	560,000,000	447,000,000	243,000,000	237,000,000
Thirty days				
1	700,000,000	420,000,000	280,000,000	230,000,000
2	580,000,000	420,000,000	250,000,000	200,000,000
3	700,000,000	400,000,000	250,000,000	210,000,000
Average........	660,000,000	413,000,000	260,000,000	231,000,000

bacteria, due to an increase in the complexity, is only an apparent
one and that the actual number of bacteria is just as great as in
the soils containing a less complex flora.   This view might be
explained on the ground that in the more complex flora there
was a larger percentage of bacteria which were not able to grow
on agar plates.   If in two soils each of which actually contained
300,000,000 bacteria per gram but in one 90 per cent of the
organisms were able to develop colonies on agar plates while in
the other only 50 per cent had this property, the counts obtained
would be 270,000,000 and 150,000,000 respectively.   Since growth

on agar was the chief means employed to obtain bacteria free of protozoa, there can be no doubt but that the protozoa-free soil used in these experiments contained a higher percentage of bacteria capable of development on agar plates than did the normal soil.

The relation of the number of kinds of bacteria in soil to the total number is in itself a large problem. Whatever the explanation for the variation in the numbers of bacteria, as determined by the plate culture method, with the complexity of the flora may be, it appears very clear that the differences in the numbers of bacteria in the soils with and without protozoa obtained in the foregoing experiments were due in large part, at least, to the complexity of the bacterial flora itself. This casts doubt upon the belief that protozoa act as a limiting factor, but it is possible that the reduction in bacterial numbers in group D was in part due to these organisms.

The effect of temperature upon the development of bacteria in soils with and without protozoa

If the soil protozoa act as a limiting factor upon the bacteria it should be possible to demonstrate that fact by the subjection of soils with and without protozoa to conditions that would inhibit the growth of the protozoa but not that of bacteria. Russell and Hutchinson explain the fact that the soil contains more bacteria during the winter and early spring months than in the summer on the view that the protozoa are not active at such low temperatures. It seems, therefore, that observations on the development of bacteria in soils which contain protozoa and others free of protozoa when exposed to winter weather should throw some light on the subject.

Four pots of sterilized soil were inoculated, two with bacteria alone and two with ordinary soil. The soils were kept at laboratory temperature for two months after inoculation and then placed out of doors during the months of December, January, February and March. The soils remained frozen throughout the greater part of this period. Bacterial counts were made just

before the pots were put out of doors and at the end of 45 days and 105 days periods outside. The data obtained are presented in Table VIII.

If the soil protozoa have a detrimental effect upon the bacteria we should expect the number of bacteria in the pots inoculated with ordinary soil to rise very markedly, while in the soils free of protozoa there should not be such an increase. There was, apparently, no difference in the behavior of the bacteria in the different soils.

TABLE VIII

Effect of low temperature upon the number of bacteria in soils containing protozoa and free of protozoa

Before being placed out doors

POT	WITHOUT PROTOZOA		WITH PROTOZOA	
	Bacteria per gram	Average	Bacteria per gram	Average
1 2	300,000,000 165,000,000	232,000,000	77,000,000 74,000,000	75,500,000

Forty-five days after being placed out doors

1 2	250,000,000 270,000,000	260,000,000	130,000,000 60,000,000	95,000,000

One hundred and five days after being placed out doors

1 2	280,000,000 336,000,000	308,000,000	100,000,000 120,000,000	110,000,000

Another experiment was performed on the relation of temperature to the bacterial flora in the presence and absence of protozoa. Three pots of sterile soil were inoculated with normal soil while three other pots were inoculated with the protozoa-free soil. One pot of the soil from each lot was then incubated at each of three temperatures, 10°C., 22°C., and 37°C. for a period of 30 days. In this case also there should be a difference in the development of the bacteria in the two soils at the various temperatures if soil is possessed of a detrimental biological factor. The results (Table IX), as in the foregoing experiment, give no evidence of a phagocytic agent.

TABLE IX

Number of bacteria in soils kept at different temperatures

INOCULUM	NUMBER OF BACTERIA PER GRAM		
	10°C.	22°C.	37°C.
Without protozoa..............	460,000,000	360,000,000	210,000,000
With protozoa.................	110,000,000	220,000,000	150,000,000

In the soils kept at 37°C. there was the same difference between the numbers of bacteria in the soils containing and free of protozoa as in the soils incubated at room temperature. The greatest difference was found in the soils incubated at 10°C., which fact is opposed to the protozoan theory, unless it be assumed that the protozoa act better at low temperatures. Such an assumption is not in accord with the known facts concerning them.

Effect of moisture content upon bacteria in soils with and without protozoa

It is generally acknowledged that protozoa require a larger amount of moisture than bacteria. An experiment was made with soils of a very low moisture content in order to eliminate the action of the "detrimental factor," if such exists in the soil. Soil with an optimum moisture content of 18 per cent was dried so as to contain only 8 per cent water. This soil was sterilized

TABLE X

Number of bacteria in soils of low moisture content

POT	INOCULUM	NUMBER OF BACTERIA PER GRAM		
		30 days	45 days	60 days
1	Without protozoa	590,000,000	660,000,000	484,000,000
2	Without protozoa	770,000,000	654,000,000	390,000,000
Average.....	Without protozoa	680,000,000	657,000,000	437,000,000
3	With protozoa	270,000,000	170,000,000	70,000,000
4	With protozoa	470,000,000	150,000,000	120,000,000
Average......	With protozoa	370,000,000	160,000,000	95,000 000

and then reinoculated, one set without protozoa and the other set with protozoa. The moisture content was held at 8 per cent and samples were taken for bacterial analysis at the end of 30, 45 and 60 days. The results are given in Table X. These show that the relation between the number of bacteria in the presence and absence of protozoa is the same in soils of low water content as when more moisture is present. This fact argues strongly against the protozoan theory.

The development of bacteria in soils containing protozoa and free of protozoa

As is well known, protozoa do not multiply as rapidly as bacteria. It is also the contention of Russell and Hutchinson that the biological factor, which they believe to exist in soils, requires much more time to develop in soil than is necessary for the bacterial flora. If such a factor exists in the soil there should be a difference in the development of bacteria in soils containing protozoa and soils free of protozoa. We should expect the bacteria to multiply very rapidly in each soil and to attain about the same maximum numbers. At a later period, in soil containing protozoa, the number of bacteria should diminish very appreciably, due to the development of the "detrimental factor," while the number of bacteria in the protozoa-free soil should remain much nearer its maximum. It has been observed on a number of occasions that the difference in the number of bacteria which develop in the soils inoculated with normal soil and with protozoa-free soil is apparent fifteen days after inoculation, and also that the difference in the two flora apparently remains the same from the fifteenth day through the third month. (Tables V, VI, VII and VIII.) The harmful factor, if such exists, must develop within fifteen days after the soil is inoculated.

Two pots of a rich garden soil, two of a field soil (loam) and two of a poor sandy soil were sterilized. One pot of each was then inoculated with normal soil and the other pot from each set inoculated with protozoa-free soil. Bacterial counts were made every day for sixteen days. The data obtained are tabulated in Table XI.

A study of the data fails to give any evidence that the proto-
zoa act as a limiting factor upon the soil bacteria. In general
it will be seen that curves representing the numbers of bacteria
throughout the period would run nearly parallel in the different
soils. The difference in the numbers of bacteria in soils with
and without protozoa was apparent from the start, which fact
indicates that the phenomena is due to a difference in the bac-

TABLE XI

The development of bacteria in soils containing protozoa and in soils free of protozoa

| | NUMBER OF BACTERIA IN MILLIONS PER GRAM | | | | | |
| | Garden soil | | Field soil | | Sandy soil | |
DAYS	A	B	A	B	A	B
1	150	40	350	250	12	15
2	200	30	315	115	12.8	8
3	400	50	400	250	40	12
4	430	275	800	200	38	12
5	1,400	420	600	400	55	25
6	1,300	620	600	410	65	20
7	1,420	760	780	500	50	27
8	1,650	925	900	450	48	21
9	950	770	910	425	36	28
10	830	430	450	220	28	12
11	620	440	530	330	40	14
12	770	360	640	275	32	12
13	1,050	490	730	215	23	9.8
14	1,030	307	617	214	24.2	11.9
15		230	385	175	31.2	11.2
16	845	257	367	204	19.4	7.8

A = soil without protozoa.
B = soil with protozoa.

terial flora and not to the protozoa. There was no evidence
that a detrimental factor developed during the latter part of
the period.

Discussion

An examination of the data presented in the foregoing experi-
ments reveals the fact that in all of the tests the soils which
contained protozoa gave lower bacterial counts as determined

by the plate culture method. This observation supports the theory of Russell and Hutchinson. It must be remembered, however, that the bacterial flora contained in the soils with and without protozoa were quite different; the flora of the protozoa-free soil was not so complex as that of ordinary soil. It was shown that, *under the conditions of these experiments*, the apparent number of bacteria was affected appreciably by the number of kinds of bacteria present. That the difference in the two flora was an important factor in the disparity between the apparent number of bacteria in the soils with and without protozoa can hardly be doubted; the assumption that it was the only factor, however, would not be justified.

If the bacterial flora in these tests was limited by the protozoa, these organisms must be able to grow actively at low temperatures (0°C.), at high temperatures (37°C.), and in soils of very low moisture content (Tables VIII, IX, and X). It would also have to be acknowledged that the harmful protozoa are capable of multiplying as rapidly in soil as the bacteria (Table XI). Such characteristics would be different from those of any protozoa now known. Admitting these possibilities, it must be concluded that the weight of the evidence is opposed to the view that the soil protozoa are harmful to the bacteria.

An objection that may be raised to this work is that sterilized soil was used as a substratum. It may be claimed that the particular kinds of protozoa which are believed, by some workers, to act unfavorably in the soil are not capable of development in sterilized soil. Such a possibility must be recognized, though there is no reason for this belief, while the evidence we have on this subject (see Part III) is opposed to such a view.

A very essential part of this work is the study of the bacterial processess in soils with and without protozoa. This phase of the work was undertaken by another worker in this laboratory the results of whose work will probably be ready for publication in the near future. It may be stated now, however, that the data obtained are in perfect accord with the foregoing observations.

V. THE EFFECT OF PURE CULTURES OF PROTOZOA UPON THE SOIL
BACTERIA

Isolation of protozoa

The term "pure culture" as it is here used in connection with protozoa means only an "animal-pure culture," since the cultures of protozoa were not obtained free from bacteria. The soil amoebae, as has been shown by Beijerinck (1896), (1897), Celli (1896), Tsujitani (1908), Frosch (1909), and others, may be cultivated on solid culture media and so can be isolated in the pure state more readily than the other types of protozoa. With the ciliates and flagellates the problem of obtaining cultures free of bacteria is more difficult. Berliner (1909) and Nägler (1909) have shown that certain of the flagellates and ciliates will grow upon agar plates but this method has not given general satisfaction. Many methods have been tried, some of which have been partially successful, but none have proved very practical.

It was found in this work that animal-pure cultures could be conveniently obtained by the dilution method. As was noted earlier in this paper in high dilutions of soil (1/10,000 gm.) the protozoa present are usually restricted to a few types. By inoculation of a large number of high dilutions of soil (1/10,000 − 1/50,000 gm.) into soil extract a few cultures which contain only one type of protozoa will usually be obtained. In order to isolate types which do not occur in soil in very large numbers it is necessary to utilize an enrichment medium which favors the growth of the desired organism. When the desired type has obtained the ascendency, it may be readily isolated by the dilution method. Hay extract, Ashby's solution, Giltay's solution, dilute peptone broth, and a weak beef extract solution have been used successfully for enrichment cultures.

After the protozoa were obtained in animal-pure cultures it was attempted to simplify the bacterial flora contained in them. This was accomplished by passage through several successive subcultures in Ashby's solution. The types of bacteria capable of development in this medium are somewhat limited.

Methods

The effect of the various protozoa isolated upon soil bacteria was tested in soil extract and then in soil. The soil tests were made by the addition of the protozoan cultures to sterilized soils reinoculated with protozoa-free soil. Tumblers each containing 200 grams of soil were used for these experiments, and the soils were incubated 30 days at laboratory temperature, which ranged from 20° to 25°C. The moisture contents of the soils were held as closely as possible to the optimum for the growth of plants and micro-organisms. (The optimum moisture content is generally considered to be very close to the moisture equivalent of the soil.) Inoculations of the soil and soil extract cultures were made by the addition of one cubic centimeter of an active soil extract culture of the protozoan under investigation.

The experiments reported here include the tests which have been made with six types of protozoa, the ciliates, *Colpoda cucullus*, and *Balantiophorus elongatus*, and the four flagellates which have been previously mentioned (Part II) as the most abundant types in soil:—*Monas sp.*, *Dimorpha radiata* (?) and two others designated for convenience as *flagellates A* and *B*.

Results with Colpoda cucullus

In soil extract, as would be expected, the number of bacteria is greatly reduced in the presence of *Colpoda cucullus*. In soil, on the other hand, no such limiting action could be detected, which further substantiates the view that this organism is not active under ordinary soil conditions.

TABLE XII

Effect of Colpoda cucullus upon the number of bacteria in soil extract

	NUMBER OF BACTERIA PER CUBIC CENTIMETER		
	2 days	5 days	8 days
Control........................	100,000,000	140,000,000	144,000,000
Control........................	128,000,000	142,000,000	100,000,000
Colpoda.......................	102,000,000	9,400,000	5,000,000
Colpoda.......................	110,000,000	9,500,000	4,000,000

TABLE XIII

Effect of Colpoda cucullus upon the number of bacteria in soil

Test 1

POT	CONTROL	AVERAGE	C. CUCULLUS	AVERAGE
1	470,000,000	490,000,000	405,000,000	452,500,000
2	510,000,000		500,000,000	

Test 2

1	1,640,000,000	1,900,000,000	2,640,000,000	2,570,000,000
2	2,160,000,000		2,500,000,000	

Test 3

1	620,000,000		770,000,000	
2	670,000,000	722,500,000	730,000,000	752,500,000
3	820,000,000		810,000,000 .	
4	780,000,000		700,000,000	

Results with Balantiophorus elongatus

The results obtained with *Balantiophorus elongatus* are similar to those with *Colpoda cucullus;* in liquid cultures the bacteria are definitely suppressed while in soil no such action is evident. The slight difference noted in Table XV is easily within the limits of experimental error. Only one test of *B. elongatus* in soil is here reported; since this organism is not active in soil (Part III) it is obvious that it could not function as a bacterial limiting factor.

TABLE XIV

Effect of Balantiophorus elongatus upon the number of bacteria in soil extract

	NUMBER OF BACTERIA PER CUBIC CENTIMETER		
	3 days	5 days	8 days
Control......................	114,000,000	141,000,000	122,000,000
B. elongatus..................	136,000,000	60,000,000	9,000,000

TABLE XV

Effect of Balantiophorus elongatus upon the number of bacteria in soil

POT	CONTROL	NUMBER OF BACTERIA PER GRAM		
		Average	B. elongatus	Average
1	620,000,000		700,000,000	
2	670,000,000	722,500,000	580,000,000	612,500,000
3	820,000,000		520,000,000	
4	780,000,000		650,000,000	

Results with Dimorpha radiata

Dimorpha radiata is an active inhabitant of soil and should therefore have some function therein. From the data obtained that function does not appear to be the destruction of bacteria. Neither in soil extract nor in soil did there appear to be any limiting action upon the bacteria.

TABLE XVI

Effect of Dimorpha radiata upon the number of bacteria in soil extract

	NUMBER OF BACTERIA PER CUBIC CENTIMETER		
	2 days	5 days	8 days
Control..........................	100,000,000	140,000,000	144,000,000
Control..........................	128,000,000	142,000,000	100,000,000
D. radiata......................	146,000,000	120,000,000	100,000,000
D. radiata......................	190,000,000	90,000,000	116,000,000

TABLE XVII

Effect of Dimorpha radiata upon the number of bacteria in soil

Test 1

POT	NUMBER OF BACTERIA PER GRAM			
	Control	Average	D. radiata	Average
1	1,360,000,000		2,050,000,000	
2	1,220,000,000	1,290,000,000	1,610,000,000	1,830,000,000

Text 2

1	470,000,000		310,000,000	
2	510,000,000	490,000,000	585,000,000	447,500,000

Results with *Monas sp.*

In the case of *Monas sp.* very interesting results were obtained. This organism, as has been shown, is active in soil. The findings reveal a very strikingly harmful effect upon bacteria in soil extract while in soil this action did not appear to take place. The reason for this is not clear, but it has been noted within recent years that the behavior of micro-organisms apparently differs, in many cases, in soil and in solution.

TABLE XVIII

Effect of Monas (sp.?) upon the number of bacteria in soil extract

| | NUMBER OF BACTERIA PER CUBIC CENTIMETER | | |
	2 days	5 days	8 days
Control........................	100,000,000	140,000,000	144,000,000
Control........................	128,000,000	142,000,000	100,000,000
Monas (sp.?)...................	200,000,000	6,500,000	5,500,000
Monas (sp.?).	46,000,000	6,800,000	6,000,000

TABLE XIX

Effect of Monas (sp.?) upon the number of bacteria in soil

Test 1

| POT | NUMBER OF BACTERIA PER GRAM | | | |
	Control	Average	Monas	Average
1	155,000,000		105,000,000	
2	105,000,000	133,300,000	290,000,000	165,000,000
3	150,000,000		100,000,000	

Test 2

1	440,000,000		380,000,000	
2	360,000,000	420,000,000	240,000,000	293,300,000
3	460,000,000		260,000,000	

Test 3

1	240,000,000		240,000,000	
2	170,000,000		165,000,000	
3	160,000,000	204,000,000	220,000,000	221,000,000
4	245,000,000		260,000,000	

TABLE XX

Effect of seven different strains of Monas (sp.?) upon the numbers of bacteria in soil

POT	NUMBER OF BACTERIA PER GRAM			
	Control	Average	Monas	Average
1	840,000,000		780,000,000	
2	930,000,000		660,000,000	
3	660,000,000		760,000,000	
4	800,000,000	786,000,000	930,000,000	788,600,000
5	700,000,000		940,000,000	
6			710,000,000	
7			740,000,000	

To verify the results of Table XIX, another test was made in which seven strains of the organism, isolated from as many different soils, were employed. A comparison was then made with five control pots which were free of protozoa. The results from this test (Table XX) agree entirely with the data given in the preceding tables.

Results with Flagellate A

This organism, like *Dimorpha radiata*, does not appear to be antagonistic to the soil bacteria as is shown by the tests both in soil and in soil extract.

TABLE XXI

Effect of Flagellate A upon the number of bacteria in soil extract

	NUMBER OF BACTERIA PER CUBIC CENTIMETER	
	3 days	5 days
Control..	57,000,000	31,000,000
Control..	77,000,000	35,000,000
Flagellate A.................................	95,000,000	31,000,000
Flagellate A.................................	58,000,000	50,000,000

TABLE XXII

Effect of Flagellate A upon the number of bacteria in soil

Test 1

POT	NUMBER OF BACTERIA PER GRAM			
	Control	Average	Flagellate A	Average
1 2	1,530,000,000 1,510,000,000	1,520,000,000	2,660,000,000 2,230,000,000	2,445,000,000

Test 2

1 2	825,000,000 900,000,000	862,500,000	725,000,000 645,000,000	685,000,000

Test 3

1 2	136,000,000 122,000,000	129,000,000	136,000,000 146,000,000	141,000,000

Results with Flagellate B

The organism used in this test, designated as *Flagellate B*, is an active inhabitant of the soil, in which it occurs in numbers nearly as great as the three preceding flagellates. This organism does not appear to affect the number of bacteria, as determined by the plate culture method, either in soil nor in soil extract cultures.

TABLE XXIII

Effect of Flagellate B upon the number of bacteria in soil extract

Period of incubation five days

CUL-TURE	NUMBER OF BACTERIA PER CUBIC CENTIMETER			
	Control	Average	Flagellate B	Average
1 2	23,000,000 43,000,000	33,000,000	39,000,000 37,000,000	38,000,000

TABLE XXIV

Effect of Flagellate B upon the number of bacteria in soil

POT	NUMBER OF BACTERIA PER GRAM			
	Control	Average	Flagellate B	Average
1	240,000,000		195,000,000	
2	170,000,000	203,800,000	165,000,000	185,000,000
3	160,000,000		210,000,000	
4	245,000,000		170,000,000	

Mixture of Protozoa

It was thought that, although none of the individual protozoa which were tried in pure culture were able to decrease the number of bacteria in soil to a measurable extent, this might be accomplished through the combined action of all of them. Two experiments were performed in which a comparison was made of protozoa-free soil and of soil containing the six types of protozoa used in the preceding tests. For these tests the soils were allowed to incubate for two months as it might be contended that one month was not sufficient time to allow the "detrimental factor" to develop. These trials, in keeping with all of the tests which have been made with the individual organisms, gave wholly negative results.

TABLE XXV

Effect of mixture of protozoa upon the number of bacteria in soil

Test 1

POT	CONTROL	AVERAGE	PROTOZOA	AVERAGE
1	250,000,000		444,000,000	
2	390,000,000		372,000,000	
3	270,000,000	294,800,000	384,000,000	357,200,000
4	290,000,000		336,000,000	
5	274,000,000		250,000,000	
		Test 2		
1	180,000,000		180,000,000	
2	240,000,000	193,300,000	176,000,000	176,000,000
3	160,000,000		172,000,000	

The conclusion of this paper with bibliography will appear in the next number of the Journal of Bacteriology.

A CULTURE MEDIUM FOR MAINTAINING STOCK CULTURES OF THE MENINGOCOCCUS[1]

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The maintenance of certain pathogenic bacteria upon artificial culture media is sometimes attended with great difficulty. Among these organisms the meningococcus may be said to occupy the first place. Its peculiar biology—particularly its intra-cellular ferment which is so potent a factor in its destruction—makes its viability at all times precarious. Furthermore its highly parasitic nature requires highly complex substances such as those upon which it grows in the human body.

While strains of the meningococcus that have been accustomed to artificial cultivation may be maintained upon plain nutrient agar, this medium is not favorable to its continued cultivation; the addition of glycerine offers no advantage; glucose results in more rapid growth and consequently more rapid degeneration. In their early work Councilman, Mallory and Wright used Loeffler's blood serum for both isolation and maintenance. Flexner used plain agar to which sheep serum was added. Some authors have used the serum of other animals—horse, goat, calf. Human serum and human ascitic fluid are conceded to be superior for isolation and for obtaining massive growth. The addition of the whole blood is possibly better than serum alone. Kutscher recommends a medium prepared with human placenta to which is added calf serum and glucose. For isolation Conradi used the centrifugalized spinal fluid, adding one part of the supernatant liquid to three parts of slightly alkaline nutrient agar; upon this solidified medium he planted the sediment.

Fluid media offer no advantage over solid media and they are of course not adaptable for isolation. Gelatin is not suitable because the meningococcus does not grow at low temperatures.

While many of these media, offer satisfactory conditions for growth during a few generations, a fair proportion of strains kept upon them suddenly fail to develop and in spite of persistent effort cannot be resuscitated. Furthermore on all the above mentioned media the cultures under ordinary conditions must be transplanted at short intervals—not longer than two or three days—and kept constantly in the incubator at 37.5°C. (Exceptional strains are found which are unusually hardy and seem to require little more care than the common saprophytic bacteria.)

With all the above culture media, those favoring the rapid growth of the meningococcus at the same time result in rapid ferment production and consequently rapid death of the culture.

After innumerable trials we have found a medium which permits of relatively slow growth of the meningococcus with apparent suppression of ferment formation, thus resulting in greater viability. This medium has been in use for about two years and its superiority over the other media mentioned in the literature for the maintenance of stock cultures of the meningococcus seems to warrant its publication. This culture medium is a modification of the potato-blood-agar used by Bordet and Gengou for the isolation of *B. pertussis*.

Preparation of medium

1. Prepare potato extract as follows:

a. Potato peeled, cut in small pieces and washed in running water for about two hours, 100 grams.

b. Water containing 4 per cent double distilled glycerine free from acid, 200 cc.

c. Mix and autoclave for forty minutes.

d. Allow to stand over night and strain through cheese cloth.

2. Make potato-extract-agar as follows:

a. Mix in an Erlenmeyer flask: Potato extract, 50 cc.; NaCl solution 0.65 per cent, 150 cc.; agar 5 grams.

b. Heat in Arnold sterilizer until agar is melted, requiring from thirty minutes to one hour.

3. Tube without filtering, and sterilize, in autoclave for about forty minutes.

4. When wanted for use, melt the agar, cool to about 45°C. and add the desired amount of sterile defibrinated horse blood.

The amount of blood to be added depends upon whether or not the meningococcus has become accustomed to the medium.

In transplanting from another medium to this potato blood agar, a little difficulty may be experienced in getting the cultures started upon the new substratum. For this reason a large amount of the growth (not over twenty-four hours old) should be transferred to the medium containing about 20 per cent of defibrinated blood. In making the inoculation the culture should be rubbed slightly into the surface. This is incubated at 37.5°C. for about 2 days and then transplanted again to the potato-extract-blood-agar containing just sufficient blood to permit growth—that is, about 5 per cent. Subsequent transplants need not be made more often than every thirteen to fifteen days or longer, when kept at 37°C., provided that the cultures do not become too dry. In the case of cultures paraffined or sealed to prevent drying, a fair growth may be obtained after six weeks.

The meningococcus grows well at 37.5°C. At lower temperatures it will remain alive for a considerable length of time, although no growth occurs; viability may be retained at room temperature apparently as long as, if not longer than, at incubation temperature. Besides, maintenance at this temperature renders paraffining or sealing less imperative for the prevention of drying. Freshly transplanted cultures that were incubated for twenty-four hours at 37.5°C., and then kept at room temperature, showed fair growth after 4 weeks. Ice box temperature will kill most strains of the meningococcus in a comparatively short time. Cultures grown at 37.5°C. for twenty-four hours, and then transferred to the ice box, grew well after five days, but after ten days about twenty-five per cent failed to show any growth at all, 50 per cent showed scanty growth, and only 25 per cent a fair growth.

The meningococcus is an aerobic organism but like many other aerobes when first grown aerobically and then transferred to an atmosphere of hydrogen, it can be kept alive longer than when oxygen is present. Cultures of meningococci were grown aerobically for twenty-four hours at 37.5°C. and then transferred to a Novy jar, the air of which was replaced with hydrogen by means of a Kipp apparatus and a Schutte vacuum pump; the jar contained pyrogallic acid and sodium hydroxide which were permitted to mix after the air had been replaced several times by hydrogen. These cultures were then kept in the incubator; after ten weeks good growths were obtained on the first transplant.

The appearance of the meningococcus growth on potato blood agar is not very characteristic. After twenty-four hours growth at 37.5°C. the individual colony has reached the size of a small pinhead. It is gray in color, smooth and rather moist looking, of an amorphous consistency, the surface elevation varying from convex to pulvinate with border entire. With age, the color of the colony changes to dull gray, the consistency becomes tenacious, and the surface elevation more of the raised type.

Although the production of pigment by some organisms is facilitated on potato blood agar, the area of discoloration characteristic of numerous strains of streptococci,—notably the *Streptococcus viridans* and pneumococcus on blood agar, and some Gram negative cocci on glucose agar as described by Elser and Huntoon—has never been observed by us with any strain.

All of our thirty-eight strains have invariably remained Gram negative, regardless of culture medium used or age of culture. Occasionally, more frequently in old cultures, a few organisms may be seen that do resist for a time the action of the decolorizing agent and thus appear to be Gram positive. However, this is usually an indication of faulty technique or of contamination.

Arrangement in pairs is most common, although single cocci and groupings in tetrads are numerous, with certain strains especially. True chain formation has never been observed. Variations in the size of organisms in the different strains are

negligible, those of individual cocci seeming to be determined more by the age of the culture, than by the culture medium. Degeneration forms occur with all strains in quite young cultures.

The potato-blood-agar furthermore is of value for differentiating between the meningococcus and the gonococcus; on this medium the gonococcus grows only when the medium has a high blood and diminished salt content—the growth is always very scanty and the characteristic differences are immediately apparent.

BILE COMPARED WITH LACTOSE BOUILLON FOR DETERMINING THE PRESENCE OF B. COLI IN WATER

MAUD MASON OBST[1]

Irregular results have often been obtained in the past in the routine bacteriological examinations of water. The presumptive test for *B. coli* made with lactose peptone ox-bile upon a sample of water would give no indication of gas in 10 cc. quantities on one day, while another sample from the same source would show gas in 1 cc. or in 0.1 cc. quantities on the following day. It was observed that when bile which had been stored for six weeks at 1°C. was used for four samples of water from a polluted spring, and bile which had been freshly collected was used for six samples from the same spring, the former produced no gas, while the latter produced gas in 0.1 cc. quantities. Similar results were obtained with the use of bile which had apparently been carelessly collected.

In this laboratory it had been found necessary to obtain bile from an abattoir in a nearby city in 5-gallon lots, and to depend upon the workers in the abbatoir for its collection. When received at the laboratory, the bile was sterilized and stored at 1°C. until used, which was frequently for two months or longer. The above results showed that it must be collected more frequently and by one who would use proper precautions. This required one-half day's time every week of a reliable helper, and an expenditure of fifty (50) cents for three gallons. Even then, the helper met with serious difficulties in gaining access to that part of the abattoir in which he could secure the unbroken gall bladder.

These experiences led to communications with other bacteriologists,[2] all of whom expressed dissatisfaction with bile media. Prof. Edwin O. Jordan, University of Chicago, has stated, "Bile from different animals varies in composition, and this is probably one reason for the variable results obtained when this substance is added to culture media. Dried bile and bile salts have been used by various observers. In my own experience bile salts, like fresh bile, inhibited *B. coli* to some extent." Previous to the time when he made this statement, Jordan determined the "degree of inhibition" of *B. coli* by bile and reported that from one-third to one-half of the vital cells of *B. coli* were thus inhibited (Jordan, 1913). He stated also that there was no evidence that these cells were more attenuated or in any way less vigorous biologically than the others.

A résumé of the literature was then made, with regard to the origin of the use of bile as a medium. It was found that Jackson (1906) had experienced the difficulty of having *B. coli* inhibited by other organisms when he used glucose or lactose bouillon as recommended by Theobald Smith (1893, 1895). Jackson, therefore, experimented with MacConkey's bile salt agar, "Platner's Crystallized Bile," which consisted of a mixture of the two bile salts, and finally with his own medium which he made by adding 1 per cent lactose to fresh ox-bile, and which gave satisfactory results in his work.

Sawin (1907) corroborated Jackson in his recommendation of the use of lactose bile and regarded it as a satisfactory and delicate indicator of minor sewage pollutions of springs and wells.

When the necessity of finding a substitute for bile was recognized, dried bile was considered. This substance, being obtained from liquid bile, varies in composition approximately in the same manner as the original material. Biochemical laboratories overcome these variations to some extent by drying bile from large quantities of mixed liquid biles, but this precaution does not produce an entirely satisfactory product.

[2] Dr. F. L. Rector, Great Bear Spring Co., New York, N. Y.; Dr. W. W. Browne, College of the City of New York; Prof. S. C. Prescott, Massachusetts Institute of Technology; Prof. Earle B. Phelps, Hygienic Laboratory, Treasury Department.

The expense of obtaining bile salts discouraged their use, and it therefore seemed necessary to find a more uniform substance which could be easily obtained.   In regard to glucose bouillon Weston and Tarbett (1907) have reported experiments upon the comparison of lactose bile and glucose bouillon in the examination of sixty-three samples of water, showing that although glucose permitted the production of gas from a larger number of samples than bile, yet *B. coli* could be confirmed from the glucose fermentation tubes in only 13 per cent of these samples.   This result was confirmed by a small number of experiments conducted in this laboratory in 1911–1912.

Members of the Hygienic Laboratory of the U. S. Public Health Service have suggested the use of lactose bouillon and referred to satisfactory results which they have obtained from its use during the past few years.   Their suggestion was strengthened by the report given by Theobald Smith (1895) that Chantemesse and Widal looked upon gas-production in lactose bouillon as conclusive evidence of the presence of *B. coli*, and by the work of Hall and Nicholls (1914) which showed that if the percentage of lactose added to bile be increased to 15 per cent the production of gas would be more rapid.   Organisms of the *B. coli* group are the only aerobes[3] commonly found in water which will ferment lactose with the production of gas.   The few anaerobes which might be found to produce gas may be eliminated by subsequent transfers to Endo's medium.   It was, therefore, thought practical to substitute this medium for bile.

When a medium is used in large quantities, as bile usually is, comparative costs become important.   Lactose and peptone are used in equal quantities in both media.   The difference in cost of the two media depends upon the cost of the raw bile in one and the meat extract used in the other.   Meat extract at contract price costs approximately three cents for the quantity required for making one liter of nutrient broth.   The

[3] The Committee on the Standard Methods of Water Analysis, in their report read before the meeting of the American Public Health Association, held at Rochester, N. Y., in September, 1915, defined the B. coli group as including all aerobic bacteria which produce gas in lactose broth.

ox-bile requires one-half day each week of the time of the helper and fifty cents for three gallons for its collection, thereby costing approximately ten cents per liter of medium. Lactose broth can be made as desired from ingredients which do not materially change during storage.

In the experiments here reported check analyses were made upon a series of samples of water, using lactose peptone ox-bile and lactose broth. The bile was never used later than a week after collection. It was sterilized upon receipt and stored during this period at a temperature of 1°C. It was enriched with 1 per cent lactose and 1 per cent peptone, and tubed in Dunham tubes. The lactose bouillon was made from neutral nutrient broth prepared with 0.5 per cent Liebig's meat extract, 1 per cent peptone and 1 per cent lactose. This medium was compared with lactose peptone ox-bile in the examination of 191 samples of water with the following results:

No gas-producing organisms in 10 cc. quantities in either lactose or bile in 68 samples.

Gas-producing organisms in the same dilutions in lactose and in bile in 59 samples.

Gas-producing organisms in higher dilutions in bile than in lactose in 3 samples.

Gas-producing organisms in higher dilutions in lactose than in bile in 61 samples.

Lactose showed gas-producing organisms in one-half as much water as were shown by bile in 12 samples.

Lactose showed gas-producing organisms in one-fifth as much water as were shown by bile in 12 samples.

Lactose showed gas-producing organisms in one-tenth as much water as were shown by bile in 19 samples.

Lactose showed gas-producing organisms in one-hundredth as much water as were shown by bile in 5 samples.

From every sample *B. coli* was isolated from the highest dilution tube showing gas. This shows the ratio of inhibition of bile on *B. coli* when compared with lactose bouillon to be about 2:1.

In some instances only a small bubble of gas appeared in the inner tube in the bile fermentation tubes, while the production of gas in lactose filled two-thirds of the inner tube. In one instance a culture of *B. paratyphosus B.*, which showed characteristic agglutination in 1: 500, was obtained from a lactose fermentation tube.

In order to obtain a direct comparison by total counts of the growth of the *B. coli* group on lactose and on bile collected at different times and stored for varying intervals, firm substrata were prepared by adding 1.5 per cent agar to each medium. It was found necessary to exert great care in filtering the bile agar in order that the final product should be free from a precipitate which would render the counting of the colonies difficult. The bile was collected each week, sterilized, and either made into agar at once or stored at 1°C. until used. For the tests, fifteen strains of *B. coli*, with characteristics described in the accompanying table, were grown for three days at 37°C., with daily transfers in 10 cc. nutrient broth.

In recording the results, it was found that the inhibition by the bile could be shown more clearly by taking the number of colonies which developed upon the bile agar as one, and considering the multiple of this number, which expressed the count on lactose agar from the same dilutious of the original culture plated and incubated at the same time, as the "ratio of inhibition" of the bile.

The bile which was collected during the month of June (see table) was tested with the cultures two days after receipt and again some weeks later. With a few exceptions the inhibition exerted by the fresh bile was less, in amount, and more regular for the different strains than that of the same bile after storage. When the actions of the various organisms upon the individual samples of bile are considered, it is seen that the variations in the degree of inhibition are greater for the bile that was held in storage before being tested; and, in general, this variation increased with the time of storage. One sample of bile (see table, (e)) was of a pronounced red color and contained a heavy red-brown precipitate. This sample showed an inhibitive action,

which was markedly irregular and which permitted no comparison with any other sample.

The contents of three individual gall bladders, collected on different dates, were also tested. The ratios of inhibition (see table, (f)) for any one organism on the three agars are nearly uniform and on the individual bile agar the different strains showed only a slight variation.

A comparison of the average inhibitive action of each sample of bile upon the different strains of organisms shows a variation from 2.4 to 3.8, and for the freshly collected bile a ratio of approximately 2.4 for all strains.

CONCLUSIONS

In these experiments lactose bouillon used as a substitute for lactose peptone ox-bile permitted the development of about twice as many *B. coli* from water as the bile medium.

Lactose bouillon costs less in money and labor. The difficulty of obtaining pure, fresh bile puts it almost out of the reach of many workers. The stored bile is proved to show progressive deterioration. Lactose bouillon can be prepared when desired and can be made more uniform. It need contain no precipitate to clog the inner tube or to affect the activity of the organism.

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Ratios* of inhibition of B. coli on bile agar when compared with lactose agar

RATIOS OF INHIBITION BY BILE AGAR PREPARED FROM LIQUID BILE AFTER STORAGE AT 1°C. FOR

CULTURE NUMBER	DESCRIPTION OF CULTURE	100 days	44 days	2 days (a)	42 days (a¹)	2 days (b)	34 days (b¹)	2 days (b)	3 days (c)	27 days (c¹)	2 days (d)	20 days (d¹)	2 days (d)	10 days (e)	2 days	2 days (f)	2 days (f)	2 days (f)
D-10	Typical acidi lactici	1.50	3.16	2.78		2.41	3.80	2.43	2.05	3.21	3.01	3.57		4.08	2.43	3.01	2.74	2.61
D-14	Slight gas in glucose, lactose, saccharose; none in dulcite; no indol	1.83	3.66	4.06	5.50	3.78	3.66	3.01	3.65	3.93	3.96	2.45		1.82	3.01	2.26	2.30	2.30
D-17	Gas in glucose, lactose, dulcite	1.65	1.47	2.11	3.55	2.32	3.38	2.40	2.41	2.78	2.37	2.48		2.48	2.40	2.45	2.30	2.21
D-18	Gas in glucose, lactose, dulcite (dulcite alkaline)			2.03	7.29	2.54	10.1	2.48	2.00	3.54	2.84	4.08		1.91	2.48	2.50	2.77	2.00
D-20	Gas in glucose, lactose; no indol	2.50		2.74	4.27	2.40	6.60	2.51	2.40	2.38	2.70	2.91		3.54	2.51	2.44	2.60	2.49
D-28	Gas in glucose, lactose, dulcite (saccharose after seventh day); no indol	2.54	8.04	2.37	2.90	2.50	2.99	2.32	2.90	2.94	3.22	3.72		1.98	2.32	2.22	2.19	2.30
O-1	Gas in glucose, lactose, saccharose (water)	2.01	2.60	2.57	3.01	2.40	2.99	2.60	2.34	2.87	2.43	2.65		3.20	2.60	2.44	2.61	2.50
O-2	Gas in glucose, lactose; none in saccharose (feces of white mouse)	2.40	2.57	2.18	3.08	2.21	2.94	2.30	2.30	2.54	2.20	2.50		4.15	2.30	2.31	2.40	2.38
O-3	Gas in glucose, lactose; none in saccharose (feces of rabbit)	2.31	2.65	2.50	5.86	2.54	2.66	2.51	2.40	2.61	2.45	2.67		2.50	2.51	1.97	2.14	1.98
O-4	Gas in glucose, lactose.; none in saccharose (feces of white rat)	3.95	1.84	2.40	2.30	1.50	1.82	2.30	2.44	2.55	2.80	2.37		1.10	2.30	2.20	2.10	2.30
O-5	Gas in glucose, lactose; none in saccharose; no indol; (water)	2.72	2.50	2.26	2.82	2.21	2.57	2.28	2.22	2.60	2.40	2.10		5.41	2.28	2.40	2.46	2.22
O-6	Gas in glucose, lactose; saccharose (rags of pigeon)	1.67	1.86	1.02	2.00	2.01	2.00	2.12	2.37	2.50	1.98	1.05		2.00	2.12	2.20	2.00	2.08
O-7	Gas in glucose lactose, saccharose (feces of dog)	2.00	4.48	2.51	3.18	2.00	2.61	2.20	2.21	2.48	2.20	2.70		4.41	2.20	2.71	2.64	2.54
D-Cream		3.89	2.16	3.01	4.95	2.45	3.30	2.45	2.72	3.12	2.50	2.72		3.81	2.45	2.47	2.50	2.50
D-Feces		7.53	3.46	3.09	4.33	2.01	4.02	2.48	2.74	2.81	2.81	3.50		3.21	2.48	2.54	2.50	2.31
	Highest ratio	7.53	9.00	4.06	7.29	3.78	6.60		3.65	3.94	3.96	4.08		5.41	3.01	3.01	2.77	2.61
	Lowest ratio	1.50	1.47	1.02	2.00	1.50	1.82		2.21	2.38	1.98	1.05		1.19	2.12	1.97	2.00	1.98
	Average ratio	2.755	3.538	2.546	3.803	2.412	3.232		2.600	2.884	2.658	2.830		3.046	2.426	2.408	2.414	2.354

(a¹) Duplicate of (a).
(b¹) Duplicate of (b).
(c¹) Duplicate of (c).
(d¹) Duplicate of (d).
(e) Red in color, and contained a red-brown precipitate after storage.
(f) Contents of one gall bladder carefully collected.
* Obtained by making duplicate determinations on lactose agar and on bile agar in every instance and by dividing the total number of colonies developing on lactose agar after 2 days' incubation at 37° C. by the number developing on bile agar.

SOCIETY OF AMERICAN BACTERIOLOGISTS

Abstracts of Papers

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SYSTEMATIC BACTERIOLOGY

Under Supervision of S. H. Ayers

Studies on the Classification of the Colon-Typhoid Group. C.-E. A.
WINSLOW AND I. J. KLIGLER.

The committee on the classification of the colon-typhoid group,
appointed at Philadelphia, has adopted a standard series of tests for
titrable acidity, hydrogen ion concentration, milk reactions, indol
production, gelatin liqefaction and chromogenesis. This preliminary
report is based on the application of certain of these tests to 150 strains
from the American Museum of Natural History collection; each strain
being tested independently in New Haven and in New York.

Our results so far indicate that there are at least three major groups
in the colon-typhoid series, the *B. coli* group clotting milk and producing
a final high hydrogen ion concentration in glucose broth and forming
indol; the *B. aerogenes* group, clotting milk but producing a final low
hydrogen ion concentration in glucose broth and failing to form indol;
and the *B. typhi* group giving a final alkalin reaction in milk but yield-
ing a high hydrogen ion concentration in glucose broth and failing
to form indol. The *B. coli* group includes at least three types, *B. com-
munis* (indol positive, sucrose negative)—*B. communior* (indol posi-
tive, sucrose positive) and *B. acidi-lactici* (indol negative, sucrose
variable). The *B. aerogenes* group is generally indol negative and
sucrose positive and includes the gelatin liquefying *B. cloacae* as well
as the typical *B. aerogenes*. The *B. typhi* group is indol negative
and includes at least three types—*B. dysenteriae* (reaction in milk
varying back and forth about the neutral point), *B. typhi* (initial
acidity followed by alkalinity), and *B. paratyphi* (alkaline throughout).

The indol reaction as determined by the use of a tryptophane medium
with 71 strains, gave results identical with those obtained by the
use of peptone on the same strains two years ago. A positive Voges and
Proskauer reaction is correlated with negative indol and a negative
methyl red test for hydrogen ion concentration and clearly marks off
B. aerogenes as a distinct group (the high gas ratio cultures of
Rogers and Clark).

The Characteristics of Bacteria of the Colon Type Occurring in Human Feces. L. A. ROGERS, WM. MANSFIELD CLARK, AND H. A. LUBS.

A total of 113 cultures were obtained from 17 individuals and classified on the basis of carbon-dioxid hydrogen ratio, indol formation, gelatin liquefaction and the fermentation of certain carbohydrates and alcohols. All but 6 of the 113 cultures fermented glucose in the absence of oxygen with the production of almost exactly equal volumes of carbon dioxid and hydrogen.

This ratio agrees with that given by 99 per cent of the cultures obtained from bovine feces and differs radically from that given by nearly all of the grain cultures. Further agreement with the bovine feces cultures is seen in the high percentage of indol formers, the absence of gelatin liquefiers, a low percentage of carbohydrate fermenters but a relatively high ability in fermenting the alcohols.

The remaining six cultures produce nearly twice as much carbon dioxid as hydrogen and otherwise agree in a general way with the high ratio group which predominated in the grain cultures.

While this type occurred in relatively small numbers the actual number may amount to several hundred thousand in each gram of material. It is possible that the more frequent occurrence outside of the animal body of the high ratio type may be because it is more resistant to unfavorable conditions and consequently survives after the low ratio type has disappeared.

The Type of Colon Bacillus Occurring in Surface Waters. L. A. ROGERS.

A collection of 137 cultures of the colon type isolated from waters of greatly varying degrees of contamination were separated into two distinct groups. One of these included about one-third of the cultures and was evidently identical with the type which has been found to include 95 to 99 per cent of the colon bacilli of bovine and human feces. This type was found occasionally in springs in which there was no evident source of contamination but was especially abundant in rivers and streams usually considered to be polluted with sewage.

The second group which occurred in practically all waters examined was identified with a type which, while it responds to all of the usual tests for *B. coli*, occurs in feces in relatively small numbers. Cultures isolated from grains belonged almost exclusively to this type. The significance of this type in water cannot be determined but the characteristic fecal colon type can be demonstrated in polluted water with reasonable certainty.

Some Problems in Bacterial Nomenclature. R. E. BUCHANAN.

The following conclusions are reached:

1. A standing committee of the society, either the committee on classification or a new committee should be asked to consider problems of bacterial nomenclature and make suitable recommendations to the society.

2. Such a committee could study the international codes of nomenclature adopted by the botanists and zoologists and report such modifications as might be necessary to adapt them to the needs of bacteriologists.

3. They could recommend a suitable date of departure for bacterial nomenclature.

4. They could report upon the historical validity of the names that are used for bacterial groups, particularly genera.

5. They could prepare a list of recognized generic names.

6. They could seek the active cooperation of societies having the same interests, in this and other countries.

7. They could prepare resolutions to be presented at the next international botanical congress expressing the wishes of the society.

8. They could prepare a list of suitable names for the designation of chemical changes brought about by bacteria.

The Hemolytic Streptococci Found in Milk. Their Significance and their Relation to Streptococci of Human Origin. DAVID JOHN DAVIS.

The occurrence of epidemics of streptococcus sore throat having some relation to the milk supply has directed the attention of recent workers to the pathogenic properties of the various streptococci found in milk.

From supplies of milk obtained under various conditions including both pasteurized and certified milk a collection of strains was isolated and subjected to various tests. They were also compared with a collection of human hemolytic streptococci as regards their pathogenicity for animals.

Especial attention was given to the study of the property of heat resistance on account of its relation to pasteurization.

Only those streptococci were selected whose colonies were surrounded by a distinct clear zone of hemolysis on human blood agar plates (Type B, Theobald Smith).

The feebly hemolytic streptococci (type A) were often noted in the milk but were not collected and studied since the interest in sore throat epidemics has centered about the cocci with a clear wide zone.

Three hundred and twenty-eight sample specimens of bottled milk were collected from different dairies in the city of Chicago. Excepting 45 samples from one dairy which furnished certified milk all the specimens were pasteurized; and with the exception of two dairies (17 samples) the holding process was used.

The time of the year during which these examinations were made was from October 1914 to March 1915.

Blood agar (human) was used in plating and the counts were made after incubation at 37°C. at the end of 48 hours. The colonies of hemolytic streptococci were carefully noted and counted on the plates and later their identity was confirmed by further tests.

Eighty-five samples yielded on culture streptococci of the strongly hemolytic variety. The number in different samples varied consider-

ably ranging from a few hundred to several thousand per cubic centimeter. In the certified milk they were about in the same proportion as in the pasteurized samples. From one dairy in 16 specimens of milk no hemolysing streptococci were found. In all others some were found.

A study of these 85 strains of hemolysing streptococci was made as regards their morphology, cultural characteristics and certain other properties. They vary considerably among themselves. They are more resistant to heat than human strains of hemolytic streptococci. They possess little or no virulence for rabbits; therefore in all probability not for man. They rapidly acidify and coagulate milk and grow well at 20°C. They may form short or long chains but as seen in milk they often appear in pairs or a chain of few elements. While they are all definitely hemolytic (type B, Theobald Smith) the characteristics of the hemolytic zone on plates may vary in certain respects.

These milk strains are different from certain strains of hemolytic streptococci found at times in diseased udders in cows. These latter resemble the strains of hemolytic streptococci from human sources and are virulent for rabbits.

There is no reason to consider that these milk strains have any sanitary significance. The importance however of certain strains of hemolytic streptococci in relation to epidemics of sore throat makes it necessary to study carefully all such organisms in milk.

By itself the hemolytic property has no more value for identification purposes than many other characteristics and perhaps less than some. But it is of great importance on account of the practical value of the blood agar plate method as a means of initial separation of hemolytic strains from the many strains of non-hemolytic and feebly hemolytic streptococci found in milk.

Hydrogen-ion Concentrations in Cultures of Streptococci. S. HENRY AYERS.

Hydrogen-ion concentrations were determined in cultures of streptococci from the following sources: 34 from various human infections, 18 from the human mouth, 89 from milk and 60 from the udder, feces and mouth of the cow, making a total of 201 cultures.

The cultures were grown in a broth containing 1 per cent cerevisine (a dry-yeast preparation), 1 per cent peptone, 1 per cent of the test substance, and distilled water. All the streptococci grew well in this medium and it does not contain a fermentable sugar. Many of the cultures would not grow in extract broth or in infusion broth when the muscle sugar was removed by fermentation with *B. coli*.

Since the acidity may be defined in terms of hydrogen-ion concentration this was determined instead of the titrable acidity. The test substances used were glucose, lactose, and cane sugar, raffinose, mannite and inulin, and the hydrogen-ion concentrations were determined by a colorimetric method.

The hydrogen-ion concentration can be represented by the symbol P^+H and on this basis the neutral point of absolutely pure water is

P+H = 6.8 at 30°C. When P+H is less than 6.8, a solution is acid and when greater it is alkaline to pure water.

A study of a large number of cultures of streptococci seems to indicate that two limiting zones of hydrogen-ion concentration are reached, as may be seen in the table.

SOURCE OF STREPTOCOCCI	HYDROGEN-ION CONCENTRATION	
	$P^+H_{4.6-4.8}$	$P^+H_{5.5-6.0}$
34 from human infections.................................	8	26
18 from human mouth...................................	18	0
89 from milk..	79	10
60 from udder, feces and mouth of cow................	54	6

These hydrogen-ion concentrations have been found to be fairly constant and would probably be more definite if the values were determined electrometrically.

It seems evident from these results that streptococci reach more or less definite hydrogen-ion concentrations, which fact may serve to help in their classification.

The difference in the limiting hydrogen-ion concentration has not been correlated thus far with any other reactions, but it is rather striking that such a large percentage of the streptococci from cases of human infection reach only the lower zone of limiting hydrogen-ion concentration.

This difference among the streptococci, brought out by the hydrogen-ion concentration, can not be shown accurately by titration methods, since the titrable acidity depends upon the composition of the medium.

The Value of Lactose Bile for the B. Coli Presumptive Test.   JOHN W. M. BUNKER.

The use of lactose bile media for the *B. coli* presumptive test has met with criticism because of the difficulty of obtaining fresh whole bile whenever and wherever needed. It has been maintained by some investigators that the use of lactose broth without bile is as efficient as the bile medium. Others have advanced the view that the inhibitive action of bile is so great that with its use weak forms of *B. coli* are lost.

The use of dried bile in the form known as Bacto Oxgall in 10 per cent solution has given results always as satisfactory as those obtained when preparing the medium from new whole bile, in tests upon oysters, milk, cream, ice cream, polluted water, and sewage.

When used in a 5 per cent solution dried bile has provided a medium which detects *B. coli* by fermentation in higher dilutions occasionally than when 10 per cent solution or Standard Lactose Bile is employed. The 5 per cent solution has never failed to show fermentation in equally high dilutions as the 10 per cent solution or the Standard medium.

A water to which was added a small quantity of *B. coli* culture when stored for a long time and tested daily with whole bile medium, ten

per cent dried bile, 5 per cent dried bile, 2 per cent dried bile, and lactose broth, showed the presence of *B. coli* at first in all media, later only in media containing 5 per cent or less of dried bile, and finally in lactose broth alone.

A water heavily polluted with feces tested in the same manner showed gas in one higher dilution in the 10 per cent dried bile and in whole bile than in the other media containing less amounts of ox gall. This water was so bad in appearance that a sanitary examination would have been unnecessary and therefore represents an exaggerated case.

For these somewhat contradictory results the following explanation is offered. It is believed that they are really not contradictory but are typical of the results which, being reported by different workers dealing with different material for test purposes, have confused the issue for some time.

The intestine is not filled with bile but bathed with it and the environment of intestinal organisms may be regarded as one of diluted bile. It seems reasonable then to suppose that a test tube of diluted bile offers a more favorable environment for these organisms than a test tube of whole bile. Organisms which develop in the standard medium do so in spite of the concentration of bile, and the weaker ones succumb.

The view is offered that in testing highly polluted material where the presence of organisms which will develop in the presence of lactose is so great that there is danger of overgrowth of *B. coli* and the shutting off of fermentation, the inhibiting property of bile toward non intestinal organisms is desirable.

Where the danger of overgrowth of *B. coli* is not great as in mildly polluted samples, lactose broth may be as efficient and will probably be more delicate.

The danger in using lactose broth is not that too many organisms will be included by the test, but that too many may be lost by overgrowth of others.

The question which ought to engage the attention of sanitary analysts is not "Shall we use bile or shall we use lactose?" but "How much bile plus how much lactose?"

The results of experimentation upon which this paper is based indicate that a medium composed of 1 per cent peptone, 1 per cent lactose, and from 2 to 5 per cent dried bile is much more delicate in detecting intestinal organisms by the fermentation test than is our present Standard medium, and is in the majority of cases more reliable than lactose broth alone.

A Chromogenic Bacillus. FRANK L. RECTOR.

This organism was isolated from water. It is a small rod with rounded ends, measuring from two to four microns long by seven-tenths of a micron wide. Occurs singly. Is motile. Stains evenly and easily; is Gram negative. Forms no nitrites, and indol production is doubtful. Coagulates milk in twenty-four hours. Forms gas in glucose, saccharose and glycerin; no gas in lactose. Thermal death point is 66°F. for ten minutes.

Produces a red pigment at room temperature. Rapidly liquefies gelatin and produces ammonia. Is a facultative anaerobe. Group number is 221.1012701.

On the Correlation of the Voges-Proskauer and the Methyl Red Reaction. MAX LEVINE.

The methyl red reaction of Clark and Lubs was suggested as a relatively quick and simple routine test for the differentiation of the colon-aerogenes group of bacteria. This test correlates strikingly with the accurately determined gas ratio. It is desirable that the methyl red reaction should also be correlated if possible with some previously employed test in order that the valuable work of the Department of Agriculture may be adequately compared with the numerous previous investigations.

From a study of 187 coli-like bacteria, 31 of which were obtained from other investigators and 156 isolated from raw and septic sewage and from the feces of the cow, pig, horse, sheep, and man, it was found that only those organisms which gave the Voges-Proskauer reaction were alkaline to methyl red. One hundred and fifty-nine of the organisms were negative to the Voges-Proskauer test; these were all acid to methyl red, while all of the 28 which reacted positively to the Voges-Proskauer test were alkaline to methyl red. It was also observed that although 23 per cent of the organisms isolated from sewage gave these two reactions (methyl red alkaline, V.P.+), no such organisms were isolated from the feces of the horse, cow, pig, sheep, and man.

The scarcity of organisms giving the Voges-Proskauer reaction in human feces is dwelt upon by MacConkey and also pointed out by Clemesha. Coli-like bacteria which give this reaction are characteristically of non-fecal origin. They resemble, in many other characters the *Bacillus aerogenes* (Escherich) and probably represent soil forms.

TECHNIQUE

UNDER SUPERVISION OF JEAN BROADHURST

Acidity of Media. H. A. NOYES.

Media for bacteriological purposes are standardized to definite degrees of acidity or alkalinity. There are several factors which may affect the accuracy with which the reaction of the media is determined. The paper, as presented, took up several of these factors and gave results of experimental work carried out in the Horticultural Research Chemistry and Bacteriology Laboratories of the Purdue Agricultural Experiment Station. The following conclusions were reached:

1. Agar-agar and gelatine should be the best obtainable. A grade of each, such that unfiltered solutions contain no visible solid matter, is obtainable.

2. Only necessary chemicals of the highest purity should be used.

3. The resulting products of reactions that will occur when a number of compounds are put together should be understood before one attempts to make combinations of them in a medium.

4. No indicator, used for the titration of media is accurate in all cases (even phenolphthalein which is very sensitive to acids will not indicate phosphoric acid accurately when it is in mixtures).

5. Enough media should be used for titration so that inaccuracies in measuring are minimized and errors in the actual titration will not be magnified in subsequent calculations.

6. The acid equivalent of media varies with the temperature of the media and the amount of variation is dependent upon the compounds present in the media.

7. Media should be added to carbon dioxide free distilled water and titrated at the temperature at which they are to be held when organisms are growing.

8. Distilled or double distilled water does not mean carbon dioxide free water. The carbon dioxide content of distilled water will not be a constant from day to day at a given laboratory.

9. Carbon dioxide in distilled water makes it possible to have a medium titrate, with phenolphthalein, 1.0 per cent acid[1] when it is alkaline or neutral.

10. The acidity of media that has been properly made does not increase appreciably when the length of time of sterilization is increased or when repeated sterilizations are made in the autoclave.

A Culture Medium for Maintaining Stock Cultures of the Meningococcus. C. G. A. Roos.

This medium has been in use for about two years and experience has demonstrated its superiority over the other media mentioned in the literature for the maintenance of stock cultures of the meningococcus. This culture medium is a modification of the potato blood agar used by Bordet and Gengou for the isolation of *B. pertussis.*

Preparation of Medium.

1. Prepare potato extract as follows:
   a. Potato peeled, cut in small pieces and washed in running
       water for about 2 hours.................................. 100 gm.
   b. Water containing 4 per cent doubled distilled glycerine free
       from acid... 200 c.c.
   c. Mix and autoclave for 40 minutes
   d. Allow to stand over night and strain through cheese cloth.
2. Make Potato-Extract-Agar as follows:
   a. Mix in an Erlenmeyer flask—
       Potato extract... 50 c.c.
       NaCl Sol. 0.65 per cent................................ 150 c.c.
       Agar... 5 gm.
   b. Heat in Arnold sterilizer until agar is melted, requiring from 30 min.
       to 1 hour.
3. Tube and sterilize without filtering in autoclave for about 40 minutes.
4. When wanted for use, melt the agar, cool back to about 45°C. and add
   the desired amount of defibrinated horse blood.

[1] Requires 10 cc. of normal alkali per liter to make it neutral.

The amount of blood to be added depends upon whether or not the meningococcus has become accustomed to the medium. In transplanting from another medium to this potato blood agar, a little difficulty may be experienced in getting the cultures started upon the new substratum. For this reason a large amount of the growth (not over twenty-four hours old) should be transferred and about 20 per cent of blood be added to the medium. In making the inoculation the culture should be rubbed slightly into the surface. This is incubated at 37.5°C. for about two days and then transplanted again to the potato extract agar containing just sufficient blood to permit growth—that is, about 5 per cent. Subsequent transplantation need not be made more often than every thirteen to fifteen days or longer, when kept at 37°C., provided that the cultures do not become too dry. In the case of cultures paraffined or sealed to prevent drying, a fair growth may be obtained after six weeks.

Pasteurization Applied to Mold Spores. CHARLES THOM AND S. H. AYERS.

A series of experiments was devised to test the effects of temperatures commonly used in pasteurization upon the spores of pure cultures of a series of species of Penicillium, Aspergillus, Mucors, one form of Fusarium, and *Oidium lactis.* Results are summarized as follows:

1. The holder process of pasteurization in which milk was heated to 145°F. (62.8°C.), and maintained at that temperature for 30 minutes killed the conidia of every species investigated, except those of *Aspergillus repens, A. flavus,* and *A. fumigatus.* The molds which survive are only found occasionally in milk.

2. The flash process of pasteurization, where milk was heated to 165°F. (73.9°C.), for a period of 30 seconds, destroyed the spores of all the molds tested with the exception of many spores of one form and occasional spores of three more forms. At 175°F. (79.5°C.), only occasional spores of two forms developed.

3. When the heating process was performed in dry air for a period of 30 minutes at 200°F. (93.3°C.), 31 out of 42 forms of Penicillium and 7 out of 24 forms of Aspergillus were destroyed, but none of the cultures of Mucors. A temperature of 250°F. (121.1°C.) over a period of 30 minutes killed all the forms of Penicillium tried, but left an occasional living spore in one species of Aspergillus and 3 out of 6 Mucors.

The use of 0.01 Cubic Centimeter Pipettes in Bacterial Milk Analysis. JAMES D. BREW.

There are two common ways of measuring milk for counting bacteria with a microscope; one, by loops and the other by capillary pipettes. Prof. H. W. Conn, in his report on an investigation recently conducted in New York City, concludes that the wire loop as used by one of the laboratories making the counts appears to yield results as accurate as those secured by using 0.01 cc. pipettes. This conclusion is based upon comparative counts; but owing to possible wide variations in

microscopical counts, due to other factors than possible inaccuracy in measuring the quantity of milk under examination, a better means of testing is by comparing the weights of the amount of milk delivered. Twenty samples, weighed as discharged by one pipette, had a variation of 3 per cent, while under the same conditions the weights of the milk discharged from a loop varied 51 per cent.

Poorly formed tips and faulty calibration cause difficulty in securing the amount of milk desired. The tip should be a truncated cone with the flattened end a circle of about 2 mm. in diameter. Pipettes should be calibrated so as to deliver (not contain) 0.01 cc. of milk (about 0.0103 grams). This may be done by weighing the amount of milk discharged into the hollow of a clean, hollow ground microscope slide, covered to prevent evaporation. A pipette should deliver 4 to 5 per cent in excess of 0.01 cc. of mercury if this is used in calibrating, as mercury does not adhere to the walls of the pipette as do water and milk.

Pipettes should be clean, but sterilization is unnecessary. Sterilizing does not remove bacteria, and growth is stopped in quickly dried smears. Smears from ten samples of milk made by individual, sterile pipettes averaged 6150 bacteria per cubic centimeter. Smears made from the same samples by individual, clean pipettes averaged 4200 per cubic centimeter while those made from the same samples by one pipette which was recontaminated between each delivery in a high count milk and cleaned by rinsing in clean water, averaged 4600 per cubic centimeter. Smears made from the same samples by one pipette which was recontaminated between each delivery in a high count milk and rinsed in the sample of milk from which the next smear was to be made, averaged 338,900 per cubic centimeter while when smears were made in the same way but with the pipette unrinsed the average was 17,723,000 per cubic centimeter. These results indicate that for quantitative milk work, individual, sterile pipettes have no measurable advantage over individual, clean pipettes and that neither have any advantage over the use of one pipette for all samples, provided it is cleaned by rinsing in clean water. Dirty pipettes or pipettes handled carelessly. cause measurable errors.

A Simple Apparatus for Isolating Anaerobes.   ZAE NORTHRUP.

A simple apparatus for isolating anaerobes consists in a 25 cc. burette complete with rubber tubing, glass tip and pinchcock. This is filled with any desired liquid nutrient medium, plugged with cotton, the delivery tube protected from contamination by inserting it in a small test tube and the whole apparatus set up on a ring stand and sterilized.

This tube may be inoculated with any material containing anaerobes. In a comparatively short incubation period the various classes of microorganisms will adjust themselves to their optimum oxygen requirements which will be noted sooner or later by a clear zone at the upper portion of the liquid varying in depth from a few millimeters to 8 or 9 cc. in some instances.

The organisms growing anaerobically may then be drawn off and sub-cultured further in these tubes until practically nothing but anaerobic organisms will be present. These may be isolated by the anaerobic plate method.

If successive sub-cultures are to be made from the same tube it is necessary to have the rubber tubing on the burette several centimeters in length so that a pinchcock may be placed about 3 cm. above the original one, the rubber tubing cut with sterile scissors and a sterile burette tip inserted. This is necessary as after one withdrawal of liquid, aerobic organisms may grow in the liquid remaining in the tip.

Modification in Staining Technic. ZAE NORTHRUP.

. The following modification in the technic of Gram's stain is recommended for beginning students in bacteriological laboratory work. If a coverglass preparation of two morphologically different organisms, one gram positive, the other gram negative, is made on the same coverglass and stained by Gram's method, the student is enabled to get the differentiation very clearly. *B. coli* and *B. subtilis* lend themselves to this differentiation very nicely.

Amniotic Fluid as a Bacterial Culture Medium. WARD GILTNER AND L. C. LUDLUM.

Amniotic fluid is a normal transudate from the blood of the pregnant mammalian female. It is a very dilute albuminous solution containing various salts and also cellular elements in suspension. While its composition varies with the different species of animals and with the stage of pregnancy it was believed that its composition was sufficiently constant and quite probably, qualitatively and quantitatively, in conformity with the food requirements of many parasitic bacteria. In fact it was believed that on account of the ease of collection and the large quantities available, bovine amniotic fluid would serve as a substitute for, or perhaps have advantages over ascitic fluid or blood serum media. At all events amniotic fluid need not be collected aseptically since it can be sterilized either in the autoclave or by Tyndall's method.

In our experiments amniotic fluid has been used in place of broth with no additions, and with agar and gelatin or with glycerine. With the colon-typhoid group, *B. coli* gave excellent growths, *B. typhi* grew scantily and *B. cholerae-suis* only fairly well. *Staph. aureus* grew best on glucose agar and on glycerine amniotic agar. None of the strains of *Streptococcus pyogenes* studied grew well except in bouillon. The growth was least on the solid amniotic agar which is a fact of considerable interest since, in the few tests conducted, *B. diphtheriae* from swabs could be isolated readily on the amniotic agar. The isolation of new strains of *B. diphtheriae* on amniotic agar was facilitated by the inhibiting action on streptococci. Involution forms of *B. diphtheriae* were not so abundant on amniotic agar as on Loeffler's blood serum medium. An old culture of *B. tuberculosis* grew with

marked vigor. For a number of years we have been satisfied that plain amniotic agar was a very nearly ideal medium for *B. abortus* (Bang).

Study of Effect of Dilution Water on Bacterial Suspensions. H. M. WEETER.

A series of tests were made to determine the quantitative changes in bacterial suspensions held in dilution water. From suspensions held for two hours at room temperature triplicate plates on lactose agar were made at fifteen minute intervals.

Of fourteen tests made with dilutions of one part milk in one hundred thousand parts water, five showed decreases ranging from ten to seventy per cent in two hours, seven showed no definite change, and two gave increases of thirty-two and thirty-nine per cent.

Since colonies of the lactic acid type seemed to be the ones disappearing thirty-two tests were made with milk inoculated with these organisms in dilution water from five different sources. The same dilution was used as before. Thirty of these tests gave unmistakable reductions in numbers, amounting in some cases to fifty per cent in fifteen minutes.

Additional tests of dilutions below one to one thousand made with milk containing a mixed flora did not show any decrease during two hours, but an increase was observed on longer standing.

The nature of the organisms present and the amount of material added to the dilution water from the original bacterial medium are probable factors determining the effect of dilution water on the bacteria present.

Variation in Plate Counts Under Research Conditions. M. J. PRUCHA.

Results of seven experiments are presented in this preliminary report. In each experiment about one hundred lactose agar plates were prepared using the same dilution of milk.

Further study is needed to give sufficient basis for drawing definite conclusions, but the results so far point to the conclusion that the average of three plates from the same dilution approaches reasonably closely to the average of a hundred plates made from the same dilution, when that average is between one and two hundred colonies per plate.

Another Use of the Double-Plate Method. W. D. FROST AND FREDA M. BACHMANN.

This method was used by Frost in 1904 to study antagonism among bacteria. It was slightly modified, renamed and used by Churchman in 1912 to study the bactericidal action of aniline dyes.

It is here modified to obviate the necessity of using either a glass or metal division by putting in a petri dish, before sterilization, a semi-disc of cheesecloth. The bottom of the dish is entirely flooded with the medium to be used and when hard the piece of cloth with the adherent agar is lifted out with sterile forceps. The clear portion of the dish is flooded with the medium containing the material to be studied. Streaks of the organism to be used are then made over each side of the

dish. In this way the agar on both sides of the dish is in perfect contact which makes diffusion readily possible.

These plates are being used to study the effect of spices and condiments in inhibiting yeasts, molds, and bacteria. This method serves as a satisfactory means of determining the preserving action of these substances.

INDUSTRIAL BACTERIOLOGY
UNDER SUPERVISION OF D. H. JONES

A Possible Function of Actinomycetes in soil. H. JOEL CONN.

A comparison has been made at the New York Experiment Station between the number of Actinomycetes in sod and in cultivated soils. The samples have been taken in pairs, one from sod and one from cultivated soil, the two spots selected always being within a few yards of each other. Thirty-five pairs of samples have been taken; and a considerable variety of soil types have been sampled. The comparative counts have been made by means of gelatin plate cultures.

Sod soil, almost invariably, has given a higher count of Actinomycetes than cultivated soil; also the Actinomycetes have comprised a greater proportion of the total flora in sod soil than in cultivated soil. In the thirty-five samples of cultivated soil, Actinomycetes comprised, on the average, 20.5 per cent of the total flora; in the samples of sod soil, 37.5 per cent, or almost twice as high a proportion. There were only three or four exceptions in the whole series, and in those the difference in favor of cultivated soil were so small as to be insignificant. The differences in favor of sod soil on the other hand have never been negligible, and sometimes have been extreme—as in one case where Actinomycetes comprised only 2 per cent of the flora in the cultivated sample but 16 per cent in the corresponding sod sample, or in another where they comprised 15 per cent of the flora in the cultivated sample, but actually as much as 60 per cent of the flora in the corresponding sod sample.

A further series of tests comparing three spots in a single soil type, one fallow, one in new sod, and one in old sod, showed 10–15 per cent Actinomycetes in the fallow spot, 21 to 25 per cent in the new sod and 37 to 39 per cent in old sod.

The most probable explanation of this difference seems to be that the Actinomycetes are active in the decomposition of grass roots, a point which is now being investigated at the New York Experiment Station.

Media for Soil Bacteria. H. A. NOYES.

In the course of other investigations in the Horticultural Research Chemistry and Bacteriology Laboratories of the Purdue Agricultural Experiment Station it was necessary to select a medium for the plating of soil organisms. The media first chosen for comparison were the Lipman and Brown agar, H. J. Conn's sodium asparaginate agar, and soil extract agars. Agar-agar alone was also used in comparison with the above media.

In plating tests the Lipman and Brown agar proved to be the best of the media studied, not only from colony counts, but in the variety of organisms grown. This latter is judged from the macroscopic appearance of the plates and by the fact that some organisms growing on the Lipman and Brown agar would not live when transferred to other media.

The colonies on the sodium asparaginate agar were large, but in only one of over fifty tests of organisms growing well on this media did the organisms fail to grow on agar alone.

The two best media were so different in constitution and the organisms that grew best on them acted so differently when transferred from one medium to the other that those ingredients furnishing carbon and nitrogen were investigated further. These tests resulted in other tests where 15 gms. of (best obtainable) agar-agar was the basis of all the media. This was used both alone, and in combination with ammonium nitrate, starch, Witte's peptone, sodium asparaginate, Liebig's extract of beef; soil extracts, wheat straw extracts, and leaf extracts were also used.

The results of this work and of later work are shown in the following table. The soil organisms reported on were chosen because of their macroscopic differences. The known pathogens and known non-pathogens were the only known organisms studied.

Media tests
(15 grams agar-agar is basis of all media.)

MEDIA	24 SOIL ORGANISMS		7 KNOWN PATHOGENS AND 8 KNOWN NON-PATHOGENS					
			Rank 3 days		Rank 6 days		Rank 8 days	
	Rank 5 days	Rank 13 days	Patho-gens	Non-Pathogens	Patho-gens	Non-Patho-gens	Patho-gens	Non-Patho-gens
Lipman plus Brown.............	3	4	12, 13	7	12	7	11	9
0.05 gram Witte Peptone........	15	15	17	16	16	16	16	16, 17
2 grams St. plus 0.05 Peptone...	6	7	10, 11	13	9	12	9	12, 13
H. J. Conn's media.............	8	9	6	8, 9, 10	6	8	6	7
1 gram Na. asparaginate........	14	14	16	14	15	13, 14	14	14
2 grams St. and 1 gram Na. asp.	9	12	8	8, 9, 10	7	9	7, 8	8
Soil plus 1 gram NH_4NO_3........	16	16	15	17	17	17	17	16, 17
Soil plus 1 gram Starch.........	13	13	14	15	10	15	13	15
Soil plus 2 grams Starch........	10	8	10, 11	8, 9, 10	11	13, 14	12	13
1 gram NH_4NO_3 plus 1 gram Starch....................	7	6						
1 gram Starch..................	11	10	9	11	13	11	10	10
2 grams Starch.................	4	3	5	6	4	5	5	5
7.5 grams Gelatine.............	12	11	12, 13	12	14	10	15	11
7.5 grams Gel. plus 1 gram Starch.	5	5	7	5	8	6	7, 8	6
7.5 grams Gel. plus 2 grams Starch	2	2	4	4	5	4	4	4
7.5 grams Gel. plus 2 grams Starch plus soil.....................	1	1	3	3	3	3	3	3
5 grams Liebig's Ext. plus 10 grams Peptone....................			2	2	2	2	2	2
5 grams Liebig's Ext. plus 4 grams Starch.....................			1	1	1	1	1	1

Are Spore-Forming Bacteria of any Significance in Soil Under Normal Conditions? H. JOEL CONN.

A series of tests has been made at the New York Experiment Station to determine whether *B. mycoides*, *B. cereus* and *B. megatherium*, the most common spore-forming bacteria in soil, occur under normal conditions as spores or as vegetative rods. Diluted soil infusion was heated at a temperature of 75 to 85°C., heated and unheated samples of the infusion plated, and the colonies of these three types on the two sets of plates counted. It was assumed that the colonies on the plates from heated infusion represented spores and that the difference in favor of the unheated infusion, if any, represented the vegetative forms.

In a series of 22 tests the average count of all three spore-formers was: 775,000 per gram, unheated; 726,000 per gram, heated. They were higher 14 times in unheated infusions, 8 times in heated. The greatest difference in favor of the unheated infusion was 530,000 per gram, which was offset by a difference of 450,000 in favor of the heated infusion; a fact which suggests that both were within the limits of experimental error. Considering the organisms separately:

B. mycoides was higher 12 times in unheated infusions, 8 times in heated.

B. cereus was higher 10 times in unheated infusions, 8 times in heated.

B. megatherium was higher 12 times in unheated infusions, 8 times in heated.

The number of spores or the total number of spore-formers present did not increase even in a pot of soil mixed with a heavy application of fresh horse manure.

These figures suggest that bacteria of this group normally occur in soil only as spores, in which form they cannot be active. This is surprising, as they are universally present, and have always been considered important. What their actual significance may be is a question.

Ferrification in Soils. P. E. BROWN AND G. E. CORSON.

Preliminary studies of the oxidation of iron in the soil, or ferrification, have shown that the process is rather a complicated one. An attempt was made to ascertain whether soils have a ferrifying power, whether such a power is bacterial or chemical in nature or the result of a combined action of several groups of factors, whether different soils have varying ferrifying powers, whether ferrification can be measured in the laboratory and finally whether the process is of any significance from the soil fertility standpoint.

Much difficulty was experienced in devising methods for the work, owing to unsatisfactory chemical methods and many series of tests were carried out merely to solve some of the chemical problems involved. A method has been devised which, although still rather crude, will permit of tentative conclusions.

Soils from a wide variety of sources were tested and it appears that ferrification is a process of common occurrence in the soil, that different

soils possess differing ferrifying powers, and that the process is in part chemical and in part bacterial in nature. A line of investigations is thus opened up which may prove of much interest. Ferrification in soils apparently should be studied further both from the technical and from the practical standpoints.

Coli-like Organisms of the Soil. B. R. JOHNSON.

Forty-two samples of soil were examined for the presence of coli-like bacteria. Eighteen samples were from manured and twenty-four from unmanured areas. It seems from this study that in both manured and unmanured soils, the incidence of coli-like organisms is considerably greater, if a crop is being raised than if the soil is fallow.

In a preliminary study of 363 cultures as to their reaction with methyl red and the Voges Proskauer test, 261 were found to be alkaline. Of these over 84 per cent reacted positively to the Voges Proskauer test. Of the 219 cultures which gave the Voges and Proskauer reaction, over 97 per cent were alkaline to methyl red. This striking correlation between the two reactions has been previously pointed out by Levine, who observes that such forms are rarely found in feces, but relatively abundant in sewage. Rogers and his co-workers found methyl red alkaline coli to be the prevailing type on grains and seldom present in cow manure. The prevailing coli-like organisms in the soil are apparently of a non fecal type and they may be differentiated from fecal strains by the Voges Proskauer and methyl red reactions.

The Influence of Soil Solution on the Longevity of Microorganisms Subjected to Desiccation. WARD GILTNER AND VIRGINIA LANGWORTHY.

It is already well known that bacteria resist desiccation in soil for a much longer time than in a naked or unprotected condition such as might be offered by the surface of a solid culture medium. It is also known that any porous substance, not in itself antimicrobial, will offer protection to microbes against the deleterious effects of desiccation. There is a question as to just what factors are responsible for the prolonged life of microbes in the soil. Soil solution, as used in these tests, was extracted by the paraffin oil displacement or pressure method. Tests were made with other solutions, viz., physiological salt, 0.1 per cent agar, gelatin, albumin, gum arabic, soluble starch, also nutrient broth and milk using *Ps. radicicola* and drying in quartz sand after suspension in the different solutions. The results were that: (a) after suspension in normal salt, gum arabic, starch or agar solutions drying in sand was rapidly fatal, few or no bacteria being alive after one month, (b) after suspension in gelatin or albumin solution drying was less rapidly fatal, (c) after suspension in milk or bouillon drying in sand was still less rapidly fatal. Suspension in soil solution followed by drying in sand gave in one case better results than with milk and all other solutions used except broth and in another case better than all other solutions except milk and broth. Tests were also made of the longevity of *Ps. radicicola* dried in quartz sand and in clay loam.

Further tests were made to determine the changes in numbers and kinds of microorganisms naturally occurring in soil solution when it is dried in different types of soils, sand, sandy loam, clay, clay loam and muck, using soil solution from a rich garden loam.

Conclusions from all the experiments were that:

1. The survival of nonspore-bearing bacteria in air-dry soil is due, in part, to the retention by the soil, of moisture in the hygroscopic form. This, however, is not the only factor, for the longevity of bacteria in a soil is not directly proportional to its grain-size and hygroscopic moisture.

2. Bacteria, at least those tested, resist desiccation longer in a rich clay loam than in sand under the conditions of our experiment.

3. The solution extracted from a rich clay loam contains substances which have a protective influence upon bacteria subjected to desiccation.

Reaction of the Soil Solution as an Index of Biological Changes in the Soil.
J. F. MORGAN AND O. M. GRUZIT.

One of the essential problems in the study of soil fertility is to adjust the reaction of the soil. This reaction essentially influences the chemical, the physical conditions and the biological life of the soil.

The junior author has found in his preliminary study of the soil solution adjusted to various degrees of reaction with N/100 mineral acid and N/100 alkali and mixed with pure sterile quartz sand, that this reaction had some effects upon the number and the type of bacteria.

An acid reaction of N/1200 had a distinct toxic action on the growth of the bacteria and the most suitable reaction for the growth of the soil bacteria was in the neighborhood of N/1000 alkali.

When changes occur from alkaline to neutral and to acid, the numbers of bacteria increase up to the point where the solution is barely alkaline and then, decrease after this point is passed. In cultures with an acid reaction, the lowering of this acidity causes the soil bacteria to increase rapidly.

The Soil Solution as an Index of the Biological Changes in the Soil.
J. FRANKLIN MORGAN.

The soil solution is a homogeneous mixture of the soil water and the soluble soil constituents, both mineral and organic.

The soil solution offers a good medium for the study of some of the biological changes in the soil. As it is the work shop of the microorganisms, this solution will contain the products of their work.

In some nitrogenous experiments with dried blood, tankage, and cotton seed meal, marked changes in the forms of nitrogen and physical conditions of the solution were noted at the different periods of extractions.

The longer periods showed a decrease in ammoniacal-N and an increase in nitrate-N. In all cases there was an increase in the total solids. This had its effect upon the physical conditions of the solution, e.g., specific gravity, specific conductance and similar phases.

The Indirect Effect of Certain Soil Treatments Upon Bacterial Activity.
P. L. GAINEY.

Different methods of preparing seed beds for winter wheat at the Kansas Station have given very large differences in the accumulation of nitrate nitrogen prior to seeding. Early (July 15) versus later, and deep (eight inches) versus more shallow plowing, have given higher nitrate contents. Efforts to trace observed differences to variations in bacterial flora have failed. Evidence was presented showing a correlation between moisture content of the surface soil and nitrate accumulation. A summary of data accumulated indicates that the various treatments have had but slight effect upon the organisms concerned in nitrate formation, except in so far as this activity is controlled by other factors.

Studies on Soil Protozoa and their Relation to the Bacterial Flora. J. M. SHERMAN.

The occurrence and activity of protozoa in soil. The results obtained from sixteen fertile soils representing various soil types indicate that these soils contain about 10,000 protozoa per gram. The predominating protozoa in the soils studied were flagellates. Ciliates and amoebae were occasionally found in numbers approximating 1,000 per gram.

It was demonstrated that certain types of flagellates are capable of multiplication in soil. The ciliates which were tested were not able to increase in soil when kept at its normal moisture content.

The effect of protozoa upon the soil bacteria. Observations made on soils containing protozoa and free of protozoa, at various temperatures, with different moisture contents, and on various types of soil indicated that the protozoa in the soils studied did not have a limiting action upon the bacterial flora.

The effect of specific types of protozoa, in animal-pure cultures, upon the soil bacteria was also studied. The ciliates limit the development of bacteria markedly in soil extract, but are not able to exert this effect in soil, since they do not lead a trophic existence under ordinary soil conditions.

Of four types of active soil flagellates which were tested three had no apparent effect upon the number of bacteria, either in soil or in soil extract. The fourth organism, a species of *Monas*, had a marked limiting action upon the bacterial flora in soil extract but apparently had no effect in soil.

The effect of volatile antiseptics upon the soil micro-organisms. The treatment of soil with volatile antiseptic does not free it of protozoa. The active soil protozoa again multiply and attain their normal numbers within one month after treatment.

The maximum numbers of bacteria in partially sterilized soils are not found while the protozoa are suppressed, but after these organisms have again reached their maximum numbers.

The number of bacteria in treated soils cannot be decreased by reinoculation with one per cent of untreated soil.

Comparisons made of treated and untreated soils under various conditions failed entirely to give any evidence in support of the theory that there exists in soil a harmful biological factor which is destroyed by the action of volatile antiseptics.

The Relation of Protozoa to Certain Groups of Soil Bacteria. T. L. Hills. In this work studies were made of the effect of protozoa on ammonification, nitrification and free nitrogen fixation in soil.

Ammonification. Three sets of the same sandy loam soil were used. One set was left untreated, another heated to 90°C. for one hour and the third heated as above and later inoculated with 1 per cent of the original untreated soil thus introducing the supposed harmful factor, the protozoa. The ammonia and nitrate determinations after 30 days revealed the following: in the untreated soil the ammonia content remained about the same while the nitrate increased slightly; in the heated soil the ammonia increased considerably but the nitrate remained the same, as the nitrifiers had been destroyed by heating the soil. In the heated and reinoculated soil the ammonia decreased slightly but there was a decided increase in nitrate formation. The protozoa introduced did not seem to have any detrimental effect on the production of ammonia and its subsequent oxidation to nitrate.

Nitrification. Flasks containing soil were sterilized by heating at 15 pounds pressure for two hours. Then one half of them were inoculated with a suspension of normal soil in sterile distilled water and the remaining half were inoculated with the same amount of protozoa free soil. This latter soil was obtained by sterilizing soil and inoculating it with as many different kinds of bacteria as could be isolated by the plate method. All flasks were then inoculated with protozoa free cultures of Nitrosomonas and Nitrobacter. Then a definite amount of ammonium sulphate was added. The ammonia and nitrate were determined after 28 days' incubation. There was but very little difference in the rate of conversion of ammonia to nitrate in the two different soil cultures.

Free nitrogen fixation. Sterile soil cultures were inoculated one half with normal soil and the remainder with protozoa free soil. Then a suspension of Azotobacter (and 1 per cent mannite in sterile solution) was added and after incubation of 21 days at 25°C. the cultures did not show any appreciable difference as regards the amount of nitrogen fixed, the difference being quite well within the limit of experimental error. In liquid cultures sterile Ashby's solution was used, one set being inoculated with soil containing protozoa and the other set with soil free from those organisms. Suspensions of Azotobacter were also added. After 21 days' incubation at 25°C. the total nitrogen analyses revealed a noticeable difference in free nitrogen fixation; those cultures free from protozoa fixing 2.05 mgs. as an average per 100 cc. of solution in excess of those containing protozoa. It seems evident that the protozoa finding here a medium suitable for their development destroyed many of the Azotobacter cells.

In conclusion it would seem from the experiments above cited that the protozoa do not have a detrimental effect on the processes of ammonification, nitrification and free nitrogen fixation in the soil.

A Study of the Nodule-Forming Bacteria. F. O. OCKERBLAD.

This paper deals with the relative longevity of *Ps. radicicola* (twelve strains from the more common legumes) in sealed and unsealed culture bottles such as are used for distribution of nodule-forming bacteria. The media used are a liquid medium (1000 cc. ash leachings from 5 grams wood ashes plus 1 per cent saccharose) and a solid medium (same as above plus 1 per cent agar).

In preparation all bottles were inoculated and incubated at room temperature (20°–22°C.) for two weeks, then half the number of each of the solid and liquid cultures of the different strains were sealed by removing the cotton plug and inserting cork stoppers which had been soaked in mercuric chlorid 1: 1000 and flamed at time of insertion. At 10-day intervals a culture of each strain on both the solid and liquid media is analyzed by plating in ash agar to enumerate the number of living cells and by making direct count with Thoma counting chamber for total numbers, dead and alive.

The bacteria in the liquid cultures both the sealed and unsealed are dying quite rapidly, with the greatest rapidity in the sealed cultures. On the solid medium the number of living cells in the sealed cultures is decreasing, approximately 25 per cent in 20 days; while the unsealed are showing little or no decrease. The total number of bacteria on the solid medium is greater than in the liquid culture media.

If we may be permitted to draw conclusions from limited and incomplete data we should say that a liquid medium for the distribution of nodule-forming bacteria is unsuitable because of the small total number and of a high mortality; and that the deterioration of cultures on solid medium in sealed form should be recognized.

Quantitative Media for the Estimation of Bacteria in Soils. R. C. COOK.

Comparative tests of several different media upon twenty soils are reported.

The length of incubation period and manner of sterilization were incidentally studied as affecting the comparative values of the respective media with the indication that five days is of sufficient duration to secure satisfactory counts.

Lipman and Brown's modified synthetic agar, Temple's peptone agar, Brown's albumen agar, and Conn's sodium asparaginate agar were compared with several other agar media having varied sources of nitrogen.

Highest counts were obtained quite consistently with the sodium asparaginate agar during the first part of the work. Later a medium was developed in which ammonium nitrate and urea were employed; this gave results fully as good as any other in a limited number of tests. Albumen agar in which the albumen was dissolved in sodium hydroxide

instead of water gave much more consistent counts, and in most cases followed closely the sodium asparaginate agar, surpassing it in one or two instances. Molds quite frequently affected the ease in counting in the albumen agar, but less so in the ammonium nitrate-urea agar. In the case of the asparaginate agar, however, there was no difficulty experienced from this cause.

It was also observed that inasmuch as all soils do not behave in the same manner toward the different media it is essential to use several soils in the comparisons; otherwise misleading results may be obtained.

Differences between the counts on the various media were not as large as might be expected and there seems to be no justification for the belief that any particular one will be most satisfactory in all cases.

Bacteria, Actinomyces, and Fungi in Soils. SELMAN A. WAKSMAN.

This investigation has been undertaken with a view to demonstrate the relationship between these three groups of microorganisms in different soils and at different depths. Soils of different texture and structure were used, and samples taken at six different depths. A six-day incubation period has been used for the counts of the bacteria and fungi and a fourteen-day period for the actinomyces counts. The results indicate that soils rich in bacteria are also rich in fungi and actinomyces. The largest numbers of all the three groups occur within the upper eight inches of the surface soil. The bacteria decrease regularly with depth, in numbers and also in percentages relative to the total numbers of microorganisms. The numbers of fungi decrease also with depth and they almost disappear below eight to twelve inches. The actinomyces numbers decrease with depth, but below eight to twelve inches their numbers remain constant up to thirty inches, and their percentage relative to the total numbers of microorganisms increases regularly with depth, because the bacterial numbers decrease, and fungi almost disappear. At a depth of 1 inch the bacteria form 81 to 86.5 per cent, fungi 6.2 to 7.1 per cent, and actinomyces 7.3 to 12.1 per cent; at 30 inches the bacteria form 16.4 to 42.1 per cent, fungi 0 to 5.6 per cent, and actinomyces 52.7 to 83.6 per cent of the total microorganic flora of the soil developing on agar plates. The actinomyces form a numerous group of soil microorganisms, especially in the ower soil depths; over 30 species of them have been isolated. The following groups of fungi occur in the soil in the largest numbers: Penicillia, Mucors, Aspergilli, Cladosporia, Trichodermae, Fusaria, and Alternaria. Many more fungus types have been isolated, but their numbers are limited.

FOOD

UNDER SUPERVISION OF CHARLES THOM

Comparison of the Number of Water Bacteria Growing on agar at 37°C. and on Gelatin at 20°C. FRED W. TANNER.

The recommendation of the Committee on Standard Methods for the Examination of Water and Sewage of the American Public Health

Association in their 1912 report, that the colony count on agar be adopted as the standard, has not met with the approval of many bacteriologists. In order to secure more data on this subject the Illinois State Water Survey began a series of comparative tests. A large number of analyses was made of which 4379 are considered in this paper.

In order to reach a more definite basis for comparison the analyses were arranged in the following classes according to their sources. The table indicates the ratios which were found. In each case the agar count was taken as unity.

SOURCE	NO. OF SAMPLES	AGAR GREATER THAN GELATIN	AVERAGE RATIO	MAXIMUM RATIO	GELATIN GREATER THAN AGAR	AVERAGE RATIO	MAXIMUM RATIO
Deep wells....................	712	54	1.1–1	25–1	658	1–1.4	1–76
Shallow wells..................	1648	148	1.6–1	17–1	1500	1–1.4	1–68
Raw Lake Michigan water.......	405	3	156–1	466–1	402	1–16	1–633
Raw river water...............	537	56	1.4–1	4.1–1	481	1–9.0	1–250
Treated water.................	1077	151	1.9–1	12–1	926	1.1–8	1–35

The relation of agar colonies to gelatin colonies on those samples showing the larger number on agar does not exceed 10 to 1, with one exception and this exception results from the consideration of only three analyses. With those samples showing the greater number on gelatin, the ratio does not exceed 1 to 10, except in one case and this again is on raw Lake Michigan water. This would seem to indicate that in badly polluted waters we might expect a high ratio, but with pure waters the counts on the two media closely approach each other.

Scientific Methods of Control in the Mineral Water Industry. FRANK L. RECTOR.

Methods of protecting the source and handling the product of the Great Bear Spring Company from the spring to the consumer are discussed. This company owns 600 acres of land comprising the entire watershed of a group of springs, 11 in number, whose flow is about one-half million gallons daily. The springs are situated five miles south of Fulton, New York.

Some 360,000 evergreen trees have been used to reforest this tract of land. Water from three of the springs is used. The springs are enclosed in enamel or glass-lined steel caissons with light-proof covers. They are perfectly protected from surface drainage.

The water is shipped in large tanks, also glass and enamel-lined, of a capacity of from seven to ten thousand gallons. Cars are sterilized by steam at 10 pounds pressure for one hour before filling. After

filling, each car is given a twenty-four hour test for gas production before being shipped.

Upon arrival at destination the car is connected by a metal hose to the storage tanks in the building and the line sterilized with steam before the car is emptied. The storage tanks are glass lined and are sterilized when empty.

Bottles are washed inside and out with hot water and soda solution, rinsed with hot sterile water, and sterilized at 104°C. for thirty minutes. When cool they are filled automatically, stoppered and sealed. Only sterilized glass stoppers are used. The piping system is sterilized daily with steam.

The laboratory work consists in checking methods of operation by frequent sampling of various parts of the system. Also frequent inspections of the different bottling houses are made, and a score card record of the visit is kept. Recording thermometers check the temperature of the sterilizers and these records are kept on file.

Analytical results show a product unchanged in the course of handling.

Bacteria in Commerical, Bottled Waters. MAUD MASON OBST.

The official supervision of commercial, bottled waters has led to the accumulation of a large amount of data concerning their bacterial content. Waters from 167 sources, both foreign and American, have been examined. Many contained large numbers of organisms, including *B. cloacae, paratyphi, mycoides, aerogenes, subtilis, aurantiacus, maritinum, ovale, prodigiosus, fluorescens (liquefaciens), fluorescens, (non-liquefaciens), M. citreus,* long-chain streptococci, and unidentified chromogens. Occasionally, common molds were found, and from one source a sporotrichum occurred in large numbers. A sample from one spring gave cultures of *P. italicum,* and from one import sample were obtained Actinomyces. *B. coli* were isolated from 57 per cent of the domestic samples and from 49 per cent of the import samples in 10 cc. quantities, from 44 per cent of the former and 28 per cent of the latter in 0.1 cc. quantities, and from 9 per cent and 3 per cent, respectively, in 0.001 cc. quantities.

In certain cases, inspections of the springs have located the sources of pollutions in some controllable place, as in the bottles or bottling houses or in a less easily controlled place, as in the spring. When the source of pollution could not be removed, the bottled product was not considered safe for human consumption.

Communications from other bacteriologists have shown that nearly all expect to find bottled waters more nearly bacteriologically pure than municipal supplies, and many feel that bottled waters should at least contain no *B. coli* in more than one of 10 cc. portions.

Comparison of Rapid Method of Counting Bacteria in Milk with Standard Method. W. D. FROST.

The method consists of making small plate cultures, four square centimeters in area, on microscopical glass slides. One twentieth of a

cc. of milk, or less, is mixed with an equal amount of nutrient agar. These "lilliputian" plates are incubated at 37°C. for from three to twelve hours, depending upon the character of the milk. The little plates are then air dried, fixed, treated with ten per cent acetic acid in alcohol, stained in Loeffler's methylene blue (1:4), slightly decolorized in alcohol, and dried. The colonies are stained a deep blue, while the background is a light blue.

The number of colonies in twenty microscopic fields is counted and the number of colonies on the entire plate calculated. This number multiplied by the dilution factor gives the number of bacteria per cubic centimeter of milk. The magnification used should be from 100 to 200 diameters. Results of thirty-seven comparative tests are given. The number of bacteria in these milks varied from 675 to 20,750,000 per cubic centimeter.

The correspondence seems reasonably close. The difference between the two counts usually amounts to less than the differences which occur between duplicate plates or the counts obtained by means of different dilutions in the same analysis, or the counts obtained on the same milk by different analysts.

The preparation of the plates requires less time than the preparation of the standard plates, the staining and counting a trifle more. No expensive apparatus is required. The amount of culture medium is very small. The time required to complete an analysis is never more than twelve hours and in many, if not most, cases can be reduced to four or five hours.

Notes on Brine Pickle Fermentation. C. W. BROWN.

In salting cucumbers there may enter the tank many types of micro-organisms; yet only those that can tolerate 12 to 20 per cent salt are concerned in the normal fermentation. The acidity of new brine is practically zero and increases gradually during two to six weeks to 50 per cent $\frac{N}{10}$ or above—a maximum of 75 to 100 per cent. The principal acids are lactic and acetic in ratio of approximately 2:1 with traces of propionic, butyric, benzoic. During fermentation gases are evolved; the volume is equal to approximately one-half the volume of the tank and consists chiefly of carbon dioxid—80 to 90 per cent; the samples contained *no* hydrogen, *no* oxygen, a trace of methane and a residual gas, presumably nitrogen. In the samples of brine analyzed alcohol was found in traces only.

The acid bacteria are facultative anaerobes, short rods or cocci arranged chiefly in chains of 2 to 5 members, they produce acid from glucose and lactose, litmus milk after a time is rendered acid but is not loppered. The ability to produce gas is questioned in that there is evidence of the strains isolated producing sufficient gas to saturate or nearly saturate the liquid medium. The gas formed during pickle fermentation is produced largely by yeasts, which can tolerate the high percentage of salt: However, motile short rods—colon type—may be isolated frequently from brine during the first stages of the fermen-

tation and these bacteria produce hydrogen in no less quantity than one part to three parts carbon dioxid.

The scum yeasts or torulae which begin to develop upon the surface of the brine during fermentation and later form a thick scum are acid consumers. In wide mouth bottles plugged with cotton the acidity of a 10-inch column of pickle brine was reduced from 74 per cent $\frac{N}{10}$ to 17 per cent in 45 days at room temperature and to alkaline 12 per cent in less than a year's time. Sterile pickle brine in test tubes inoculated with pure cultures of the scum yeasts was reduced in acidity from 35 per cent $\frac{N}{10}$ to neutral or alkaline within 30 days time at 20°C. The first acid to be consumed is the lactic, leaving the acetic until the last, that is, the ratio changes until the acetic is the predominant or only acid. Tubes of pickle brine agar or even tubes of litmus agar to which sterile commercial lactic acid is added—as much as 200 per cent $\frac{N}{10}$ (1.8 per cent pure acid)—when inoculated with these scum yeasts are rendered neutral or alkaline adjacent to the growth of the yeast within a few days' time. Under similar conditions acetic acid is consumed with difficulty.

The fermentation of brine pickles is an associative action of various microorganisms resulting in (1) the using up of those constituents of the cucumber which may be used readily as microbial food—protein made soluble, sugar changed to acid, etc.—; and (2) in preservation of the depleted cucumbers (brine pickles) in the brine containing the by-products. When the acidity which is a potent factor in preservation is destroyed from the surface of the tank downward by the scum yeasts the brine pickles are liable to decomposition.

Sampling Milk for Bacterial Analysis. ROBERT S. BREED.

In the series of comparative studies on the plate and microscopical methods of counting bacteria in milk which are in progress at the New York Agricultural Experiment Station, some tests of methods of sampling have been carried out. In these tests, comparative counts have been made in order to discover whether samples of milk taken in clean test tubes containing preservatives (formaline or corrosive sublimate) were as satisfactory for use in making microscopical counts as iced samples taken in sterile tubes. The results secured are not sufficient to warrant a positive statement but indicate that samples taken with preservatives are as satisfactory as are the iced samples and much more convenient to handle. When an effort was made to keep the preservative samples for days or weeks, it was discovered that they became less satisfactory, the longer they stood. This was not because the organisms lost their staining power or because of any growth of organisms in the samples but because the bacteria floated to the top with the cream which became compact on standing. Some of them also fell to the bottom. Because of the fact that it was impossible to shake a sample so as to break up both the cream and the sediment perfectly, the counts secured from the samples after standing tended to be lower than they should have been.

Counts made from the cream and sediment of both iced and preservative samples showed that this concentration of the bacteria in the cream and in the sediment occurred in all of the samples. Where no cream was present as in skim milk, the bacteria did not rise to the surface but sedimented in large numbers showing that the reason for their concentration in the cream was because they were buoyed up on the fat drops. In the samples studied, there was a strong tendency for the larger clumps of bacteria to concentrate in the cream, the bacterial groups which occurred in the sediment rarely consisting of more than two individuals.

The Pasteurization of Dairy By-products. ROBERT S. BREED AND W. D. DOTTERRER.

In some work done for the New York State Commission for the Investigation of Bovine Tuberculosis during the summer and fall of 1915 on the pasteurization of whey, it has been found that whey, heated between 140 and 180°F. and allowed to cool slowly in the whey tank, sours with an almost pure lactic acid fermentation due to lactic acid bacilli belonging to the *Bacillus bulgaricus* group. Immediately after heating the numbers of bacteria in the raw whey are reduced from millions to tens of thousands per cubic centimeter. During the 18 to 20 hour period which elapses before the whey is returned to the farmers and during which time it is cooling slowly, there is a rapid growth of the lactic acid bacilli which have survived the heating so that the whey contains from tens to hundreds of millions of these organisms per cubic centimeter as delivered to the farmers. The other types of bacteria present (largely spore forming bacilli) do not increase in number to any marked extent. The acidity of the whey as delivered to the farmers was found to vary from 0.3 to 0.4 per cent calculated as lactic acid.

On the other hand the unheated whey which was examined showed an acidity of 1.2 per cent and contained several million miscellaneous bacteria, one and a half billion lactic acid bacilli and about thirty million yeasts per cubic centimeter. Neither of the latter developed on the agar media and would not have been found if the microscopic method of counting had not been used. No heated whey was found which contained yeasts, a condition which suggested that the improvement in the quality of cheese frequently noted where pasteurization has been adopted has arisen from the elimination of yeasts from the whey tank and so from the farmers' milk cans.

On two successive days at one of the factories, the predominant lactic acid organism in the making vat was found to be a bacillus instead of the more common Streptococcus.

The Effect of Air Pressure on Potable Waters During Storage. W. D. FROST AND FREDA M. BACHMANN.

Steel pressure tanks are in common use for storing water. The question is raised whether or not the effect of the air under pressure in these tanks could be injurious to the contained bacteria, or, in other

words, whether these plants could be depended upon to improve the water if it came from a contaminated well.

Experiments conducted with small quantities of water (2 to 3 liters) held in steel chambers at varying degrees of pressure up to 100 pounds per square inch, and at the temperature of a warm room, showed that the contained bacteria increased very rapidly and to an enormous extent. *B. coli* was not affected by the pressure.

When similar samples were held at the temperature of an ice box, but otherwise under the same conditions, the growth was marked but slow. When water held under similar conditions in the ice chest was partially renewed at intervals of 24 hours by pouring out half the water and putting in fresh, the number of bacteria appeared to remain nearly constant.

An examination of several plants in actual operation showed that the water in these tanks remained practically constant so far as their bacterial content was concerned.

The Bacterial Content of Market Oysters.   FRED BERRY.

Eight samples of shell oysters and twenty-one samples of shucked oysters, collected from eighteen different retail markets in Chicago were examined according to the methods recommended by the Committee on Standard Methods of Shell-fish Examination. The first sample was collected October 13 and the last April 27. Additional tests were made to determine whether the use of the combined shell liquor of fifteen oysters, as recommended by Smith, would necessitate a different interpretation from that based on the analysis of five individual oysters, as recommended by the Standard Methods Committee. A few samples of shucked oysters were re-tested after storing in the ice-box for forty-eight hours to determine the character of the bacteria which multiplied most rapidly under such conditions.

Of the eight samples of shell oysters, three contained an excessive number of *B. coli*. These were collected October 13, October 23, and March 2, and had a score of 41,140, and 120, respectively. The other five samples were collected in February and four of these had a score of 0, one a score of 23, and the other a score of 5. The lowest count on the shell oysters was 2600 on a sample collected February 8, and the highest was 7,740,000 bacteria per cubic centimeter on a sample collected March 2nd.

Of the twenty-one samples of shucked oysters, none was free from *B. coli*. The minimum was 1 and the maximum 40,000 *B. coli* per cubic centimeter of oyster liquor. Fifteen of the twenty-one samples contained 100 or more *B. coli* per cubic centimeter. Eight of these fifteen contained 1000 or more *B. coli* per cubic centimeter. The count on shucked oysters varied from a minimum of 140,000 to a maximum of 34,000,000 bacteria per cubic centimeter of oyster liquor.

These results may be summarized as follows:

1. The shell oysters purchased at Chicago during February contained fewer bacteria than those purchased in October and March.

2. The season of the year apparently had little influence on the character of the bacterial content of the bulk oysters, a majority of the samples containing a very large number of bacteria, many of which belonged to the *B. coli* group.

3. No definite correlation existed between the total number of bacteria and the number of gas formers found in the samples.

4. The use of five or fifteen shell oysters for a sample did not materially affect the interpretation as to the sanitary quality of the sample when judged by the U. S. Standards.

5. On the basis of the bacteria developing on plain agar at 20°C., on Endo medium at 37°C., and the presumptive test for *B. coli*, the increase in bacteria in bulk oysters during 48 hours storage in the ice-box cannot be interpreted as being due mainly to an increase in intestinal bacteria.

Normal Fermentation of Sauerkraut. LESTER A. ROUND.

The fermentation of sauerkraut was studied in two factories. In the first factory, microscopic and chemical examinations were made while in the second factory a bacteriological study was also made. The microscopic examination showed that bacteria alone are concerned with the proper fermentation. Wherever air came in contact with the kraut or brine, as at the top of the vat, yeasts grew very rapidly after the first week and produced a heavy foul-smelling scum which rapidly destroyed the acid. Analysis of fresh juice from a vat just being filled showed the presence of five million bacteria, 80 per cent of which were glucose fermenters. The remaining 20 per cent were mainly, if not all, yeasts. The high count was due chiefly to the refilling of tanks which had just been emptied and the walls served as a means of inoculation with acid-producing organism. It was found that in the first 24 hours the plate count would go up to about 100,000,000. During the first week it would go up gradually to 200,000,000 to 300,000,000.

The rate of growth of bacteria and the rapidity of fermentation varied directly with the temperature and were much slower in cold weather than in warm. After reaching a maximum, the number of bacteria gradually decreased until at the end of five weeks there were present between four and ten million viable organisms. Lactose-bile fermenting organisms were found in small numbers at the start. These increased rapidly for the first few days and disappeared rapidly after the kraut showed an acidity of plus 7.0. These organisms probably came from the wagons, forks and shoes of the farmers who brought in the cabbage. Examination of the interior of the cabbage-head showed it to be sterile.

Vats showing abnormal fermentation contained a different class of organisms. A study of such vats indicated that bad fermentations in a properly salted vat were due to the growth of unfavorable organisms during the first few days before the normal acid flora had been able to establish itself and produce sufficient acid to stop decomposition. In the course of normal fermentation there was found to be a slight increase in the temperature.

A Study of the Effect of Spices on the Growth of Certain Organisms.
FREDA M. BACHMANN.

A study of the preservative effect of spices in foods was made in order to determine the relative efficiency of the different spices in inhibiting the growth of microorganisms. The molds used for inoculation were the common ones found on spoiled fruits and vegetables, species of Rhizopus, Penicillium, Aspergillus, and Alternaria. Of the bacteria, *B. coli, B. subtilis,* and *B. prodigiosus* were studied. A yeast isolated from Fleischman's compressed yeast was also used for inoculation. The molds and yeasts were grown on Thaxter's potato hard agar and the bacteria in the ordinary nutrient agar.

A new method for obtaining a double plate was devised in which the agar without the spice covers one-half of a petri dish and that with spice the opposite half. In this way the organisms may be grown on two kinds of media in one plate. The organisms were grown in such double plates, also in spiced agar slants, and on steamed apples to which varying amounts of spice were added. Besides the study of the effect of ground spices, the alcoholic extracts, the active principles, and the oils were used.

Cinnamic aldehyde is most effective in preventing growth of all the organisms studied. Eugenol and oil of allspice also have a considerable preservative effect. Nutmeg is of little value as a preservative and black pepper and ginger have practically no effect. It was found that there is considerable variation in the sensitiveness of different organisms. Molds were found to be more sensitive than the bacteria and yeast. There is a very considerable difference in the amount of spice necessary to prevent germination of mold spores and the amount necessary to inhibit a growth of the mycelium. The results of this study for the most part confirm those of Hoffman and Evans in their work on spices as preservatives.

SANITARY BACTERIOLOGY

UNDER SUPERVISION OF HENRY ALBERT

Influence of Conditions in the Barn Upon the Germ Content of Milk.
M. J. PRUCHA AND H. M. WEETER.

The aim in this study was to measure the collective influence of the barn conditions and operations on the bacterial contamination of milk. Pails were steamed before each milking and the samples of milk for the analysis were taken from individual cows when the pail of milk was brought out from the barn into the adjacent milk room.

The study was conducted from March to July in 1914 and 1915 in three different barns. Barn I was very clean, barn II was not as clean as barn I, and barn III was decidedly dirty. 1710 samples were taken in all. The results are summarized in the following table:

BARN	AVERAGE NUMBER OF BACTERIA OF ALL SAMPLES	
	1914	1915
I	2,288	3,229
II	1,073	873
III	6,604	5,255

Table showing the lowest and the highest counts and the average of fifteen samples from each cow, 1914

	BARN I				BARN II				BARN III		
No. of cow	Bacteria per 1 cc.			No. of cow	Bacteria per 1 cc.			No. of cow	Bacteria per 1 cc.		
	Average	Lowest	Highest		Average	Lowest	Highest		Average	Lowest	Highest
174	183	17	532	170	222	27	582	1,034	2,667	307	20,365
135	325	47	1,215	116	265	50	815	1,019	2,748	563	7,285
189	387	50	2,502	166	295	53	890	1,031	3,150	1,088	9,725
167	444	45	1,780	123	329	3	1,078	1,025	4,320	970	22,146
150	506	50	1,457	165	356	45	1,087	1,033	4,598	855	18,520
187	585	247	1,932	159	373	63	920	1,032	4,603	1,390	9,275
171	602	272	1,480	557	603	480	820	1,018	5,324	2,120	13,735
155	613	40	2,560	552	635	63	4,025	1,015	6,414	1,453	12,108
63	657	72	3,257	113	636	100	1,307	1,003	13,120	3,425	29,500
110	665	245	1,300	145	657	52	1,860	1,026	19,092	7,480	63,835
177	758	82	1,783	125	698	50	4,373				
35	723	40	5,705	550	735	30	4,862				
130	751	77	2,760	108	770	31	2,247				
176	826	332	1,323	163	813	11	2,530				
156	763	281	2,117	149	966	32	5,075				
182	833	282	3,425	553	1,045	108	2,012				
179	837	103	2,760	556	1,117	92	4,444				
26	872	140	3,800	117	1,217	70	4,025				
192	888	242	2,182	551	1,258	44	3,250				
73	927	192	3,627	554	1,292	107	4,925				
74	931	97	4,850	137	1,369	433	2,587				
178	925	412	1,686	183	1,613	225	6,612				
186	1,042	347	2,400	131	1,878	232	4,675				
152	1,044	90	8,060	175	2,425	1,362	3,450				
190	1,140	337	6,225	118	2,752	11	12,955				
134	1,164	167	3,395	555	3,588	29	33,000				
154	1,307	192	4,157								
184	1,391	135	6,275								
112	2,010	342	6,900								
191	2,213	320	10,135								
180	2,529	132	28,950								
172	3,874	937	8,505								
188	5,231	67	58,275								
111	6,835	3,095	15,812								
55	35,131	2,255	218,250								
	2,288				1,073				6,604		

Relation of Bacteriology to City Milk Standards. H. A. HARDING.

Standards presuppose something to be measured and measurements presuppose comparison.

Satisfactory city milk standards should furnish a basis for accurately comparing the various milks which may be analyzed from three essential standpoints: (1) food value, (2) freedom from disease germs, (3) cleanliness.

Food value. Bacteriology bears no direct relation to food value. It might bear an indirect one if high term content was accompanied by a lowering of food value. In commercial milk this reduction is not appreciable, except as induced acidity interferes with certain uses of milk.

Freedom from disease germs. Bacteriology has everything to do with this feature, but practically we have no method of determining the presence of such germs and protection must be sought through omnibus methods such as pasteurization. Pasteurization control is mainly through time and temperature.

Cleanliness. Added uncleanliness is probably best measured by bacteriological counts if they are made at the time of infection. However, as soon as the elements of time and temperature enter, such counts no longer indicate the character or extent of contamination.

Conclusion. Quantitative bacterial standards of 1,000,000 or any similar number do not throw any light upon two of the three elements which are important in judging a milk supply, and unless the age and temperature history of the milk is known they do not give any important information regarding the third element.

Purification of Sewage by Aeration in the Presence of Activated Sludge. EDWARD BARTOW.

By blowing air into sewage then allowing the suspended matter to settle and decanting the supernatant liquid, adding fresh sewage and repeating the operation, there is accumulated sludge which has the property of purifying sewage, in the presence of air in from four to five hours. The sludge obtained contains more nitrogen than sludge obtained by any other method of sewage purification. It has been shown by analyses and by experiments with growing plants that it is valuable as a fertilizer. By the process a bacterial reduction of 95 to 99 per cent is effected. The cost of the process depends upon the cost of producing air. It has been estimated that it will be the most effective and most economical method of sewage purification. This will be especially true if the sludge can be readily recovered and disposed of for use as a fertilizer. Plants of considerable size have been constructed at Milwaukee, Cleveland and Champaign and the process will be given a thorough trial.

Diphtheria Diagnosis by means of Blood Serum containing Potassium Tellurate. WILL SHIMER.

The medium for diphtheria cultures devised by Conradi and Troch has not been generally adopted apparently for two reasons; first, the tellurite salt instead of the tellurate salts has been used by most workers, second, the Conradi medium was first recommended as a color differentiating medium as well as an inhibiting. The color differentiation medium is now believed to be of little help.

The Bacteriological Laboratory of the Indiana State Board of

Health has used three dilutions of potassium tellurate: e.g., 1.4, 1.5, and 1.6 cc. of a 1 per cent solution of freshly made up potassium tellurate for each 100 cc. ordinary Loeffler blood serum.

Three hundred and eleven parallel diagnostic cultures on ordinary Loeffler's blood serum and the same number containing 1.6 cc. potassium tellurate per 100 cc. were made. This dilution of potassium tellurate medium gave 2 per cent less positives than the Loeffler's blood serum. Of 246 parallel diagnostic cultures on ordinary Loeffler's blood serum and the same number containing 1.4 cc. potassium tellurate per 100 cc., the dilution of potassium tellurate medium gave 1.2 per cent less positives than did the Loeffler's blood serum. Of 890 parallel diagnostic cultures on ordinary Loeffler's blood serum and the same number containing 1.5 cc. potassium tellurate per 100 cc. the potassium tellurate dilution medium gave 2 per cent more positives than the Loeffler's blood serum.

The increased number of positives obtained with the potassium tellurate medium is not by any means a measure of the complete advantage of this medium. Smears made from the potassium tellurate medium contain fewer bacteria, and their use decreases the time necessary to examine the microscopic slides almost half and lessens the work of getting pure cultures enormously.

The Number of Bacteria in the Air of Cow Stables. G. L. A. RUEHLE.

In the course of an investigation of the air as a source of bacteria in milk which has been made at the New York Agricultural Experiment Station, it was necessary to make a large number of analyses of stable air under a variety of conditions. Altogether 1130 separate analyses of air samples were made but since many of them were duplicate analyses or were made under artificial conditions only 402 analyses are summarized in the work reported upon here. Of these 344 were made in the Station stable and 58 in commercial dairy stables. The aeroscope used in the majority of cases was a simple modification of the sand filter aeroscope recommended by the Committee on Standard Methods for the Examination of Air. The modification was of such a nature as to permit dry sterilization, at the same time eliminating some of the joints where leakage might occur.

The average germ content of the air in 344 tests was 115 per liter. The lowest number of bacteria was found, as would naturally be expected, when the barn was empty and everything was quiet. Sixty analyses made under these conditions showed an average of 41 per liter. The highest average numbers were found after milking was finished and silage was being fed. This caused the cattle to move about, stirring up an evident dust. Ten analyses taken under these conditions showed an average of 271 per liter. Individual tests among the 344 analyses gave results varying from 0 to 825 per liter. The germ content of the air of three commercial stables was found to be similar to that of the Station stable except that four analyses taken

under dusty conditions occasioned by feeding hay or corn stalks gave noticeably higher figures than any of those recorded above. The results of these four analyses were 1100, 2400, 3957 and 16,070 per liter respectively.

From the foregoing results, it is evident that the air of dairy stables contains many more bacteria than have been found by Winslow and Browne (*Monthly Weather Bureau*, 42: 452–453, 1914) in country air, city street air, offices, factories and schools. This is not surprising as relatively dusty operations such as feeding dry hay, grain and the like must be carried out several times daily in every cow stable. In spite of this fact, it must not be concluded that air plays a great part, numerically, in the contamination of milk by bacteria. The studies made in order to discover the relative importance of this factor in milk contamination have shown that the air is a relatively unimportant source of bacteria in milk. The detailed results of the latter investigations are published in *Bulletin 409* of the N. Y. Agr. Exp. Sta. which has just been issued.

Validity of Presumptive Tests.   W. F. Monfort.

Each presumptive coli test proposed from time to time has been first applied locally. Its extension to other regions and other classes of waters has developed certain limitations. None has proved of universal application. It is therefore of first importance that the saving clause of Standard Methods, 1912, page 96, be given due consideration in evaluating any abridged test for the colon group before its adoption with respect to waters of a class or region new to the investigator. This discrimination is not always practiced.

There follow some results of such an evaluation with respect to a surface water (Missouri River) from which a turbidity of 1000 to 12,000 parts per million has been removed; the effluent is treated with bleach. Observations covering more than two years show this supply to contain usually not more than two organisms of the colon group per 100 cubic centimeters.

Neutral red bile-salt lactose broth gives positive results in all dilutions.

Aesculin bile-salt broth, giving negative results with *B. cloacae*, yields a brown coloration with an organism of frequent occurrence belonging to the class *B. fluorescens*.

Lactose-bile gives an error of over 73 per cent as compared with confirmation tests of lactose-fermenting, acid-forming, aerobic bacteria.

In lactose broth 80 per cent of gas formers fail of confirmation.

For a water of this class apparently nothing thus far proposed short of actual discriminatory tests, at least so far as outlined in the lately adopted "standard" for waters used on common carriers in interstate commerce, can be considered valid.

INFECTION AND IMMUNITY

Under Supervision of A. I. Kendall

A Study of the Bacteria of Normal and Decayed Teeth.　I. J. Kligler.

Material collected from deposits on teeth of 40 individuals was studied with the object of determining the numbers and types of bacteria found in such deposits, normally and at various stages of decay. Twenty specimens were taken from healthy teeth in mouths of different states of cleanliness, and twenty from carious teeth in different degrees of decay. The complete results of this investigation were published in *The Journal of the Allied Dental Societies*, 1915, vol. x, pp. 141–166, 282–330 and 445–458.

Bacterium pyogenes Associated with a Case of Multiple Arthritis in a Hog.　Archibald R. Ward.

The writer pointed out that polyarthritis of swine is a condition frequently encountered in postmortem inspection of meat. A case showing various stages of articular involvement from the early stages of synovitis to later stages showing erosion of articular cartilage, exostosis and anchylosis of the joints was subjected to bacteriological and pathological examination. *Bacterium pyogenes* was isolated in pure culture by the method suggested by Künnemann. This consists of employing agar to which has been added about 30 per cent of sterile raw cattle serum, just previous to pouring the plates. The organism was also isolated from three abscesses near a joint. The walls of two of these abscesses were in contact with the synovial membrane. The abscesses contained an odorous pus greenish yellow in color.

The synovial membrane was highly reddened and was covered with vegetations in the form of minute vascularized tufts or tassels. Sections of the membrane stained by the Gram method showed organisms similar to *Bacterium pyogenes* within certain cells.

The organism in question has been found by European investigators to be very frequently encountered in chronic suppurative conditions in both cattle and swine, observations that have been confirmed by the present writer.

Spirochaeta Hyos.—Its Antigenic Value in Complement Fixation Tests on Hog Cholera Sera. Studies on Hog Cholera.　Walter E. King and R. H. Drake.

With antigen prepared from pure cultures of *Spirochaeta hyos*, 115 complement fixation tests have been conducted up to the present time. Of these, 22 tests were with normal hog sera from 10 different animals, 1 from an animal which exhibited a reaction only following inoculation with virus, 6 tests from 2 convalescent or naturally immune swine, 84 tests with sera from 34 animals suffering from hog cholera (4 of which had been used as normals) and one test each with 2 different lots of hyperimmune serum. Negative readings occurred in all cases in which normal hog sera were subjected to complement fixation tests.

sitive readings resulted in all tests with sera from cholera hogs with 2 exceptions.

Complement fixation is coincident with clinical symptoms and depends upon the virulence of the infecting material and the individual resistance of the animal.

Tests of two convalescent hogs indicate that complement binding substances cease to exist in the blood of hogs when immunity against hog cholera becomes fully established.

Control antigens made from cultures of *B. choleras-uis*, *B. Voldagsen* (Haendel), *B. typhi-suis* (Glaesser) fail to exhibit complement fixation with cholera sera.

Antigen prepared from pure cultures of *Spirochaeta hyos* possesses no complement binding properties upon sera of hogs suffering from septicemia, *B. cholera-suis* infection, Anthrax, Ghon-Sachs infection, brine poisoning, or pneumonia from natural exposure.

We believe that, by the observance of proper technique, the results recorded herein can be duplicated without difficulty and that the method may be used to practical advantage as a reliable, accurate means of laboratory diagnosis of hog cholera. Furthermore, the results of these experiments support our former conclusions that *Spirochaeta hyos* merits serious consideration as an organism possessing specific pathogenic properties in relation to hog cholera.

Antigenic Properties of Autolysed Bacteria. GEORGE H. ROBINSON.

Meningococci were allowed to autolyse at different temperatures for varying periods of time. The filtrates and residues were tested for their complement fixing and complement absorbing properties. Only after 24 hours in distilled water at 56° is the fixing power of the filtrate greater than that of the residue. The fixing power of autolysed suspensions decreases in proportion to the extent of autolysis indicating a degradation of the protein. A small portion of the original antigenic substance is obtained in a filtrate after autolysis. A fresh, washed, bacterial suspension gives more satisfactory results as an antigen for complement fixation tests than an autolysate.

The Effect on Horses of Feed Heavily Inoculated with B. coli Isolated from Oat Hay. ROBERT GRAHAM AND L. R. HIMMELBERGER.

The occurrence of *B. coli* or colon-like organisms on grains has been demonstrated numerous times. Recently Rogers, Clark and Evans[1] published a report of their studies of colon bacteria on grains. They isolated one hundred and sixty-six cultures, of which seventy-five were obtained from corn, six from barley, thirty from wheat and forty-one from oats. The grains used were secured from the grain inspection laboratory and should therefore represent average samples grown throughout the different sections of the United States. This widespread occurrence of colon-like organisms on grains, together with the fact that *B. coli* or colon like organisms appeared to be constantly

[1] Journal of Infectious Diseases, vol. 17, no. 1, 1915.

present on oats which were proved to be the cause of a serious outbreak of a disease commonly known as "forage poisoning," suggested the possibility of some pathogenic or virulent type occurring on grains, thus explaining some of the losses occurring to the live stock industry.

To determine the effect produced by ingestion of strains of *B. coli* isolated at this laboratory, horses were fed a wholesome feed heavily inoculated with the isolated cultures of *B. coli* grown on broth and agar media. One horse received agar cultures on corn meal in addition to oats which had previously been inoculated. After four days this animal developed diarrhea, showed a sluggish attitude, and regardless of the amount of wholesome feed consumed lost in weight. A mule was fed 200 cc. of broth culture ranging from forty-eight to seventy-two hours old twice daily. In this animal loss of appetite occurred and the animal became weak and suffered from diarrhea.

A third horse was fed for eighteen days on oats heavily inoculated with *B. coli* after being frozen for four days at 30°F. and allowed to thaw slowly. This animal evidenced an indifferent appetite, was greatly depressed and lost in body weight. Another horse was fed oats which had been previously inoculated with broth cultures of *B. coli*, with the result that the animal suffered loss in weight.

In no case were we able to produce death by feeding, but the condition in the experimental horses was such as to suggest that feeds contaminated extensively with colon bacilli lower animal vitality and render the animal more susceptible to other injury. While most investigators consider colon contamination of grains the result of fertilizing soils with animal fecal matter, some believe that multiplication actually takes place on the grain. For instance, Prescott (cited by Rogers, Clark and Evans) found *B. coli* on grains grown under conditions which gave no history of contamination with fecal material. If this be a tenable view it will account for the occurrence of *B. coli* in greater numbers than can be accounted for by the theory of contamination. Since the toxins of *B. coli* have been proven by Vaughn and Cooley[2] to be intracellular, it follows that the effects observed by us must have been produced by disintegrated bacterial cells. In this connection we desire to mention the effect of daily intravenous injections of dead colon bacilli washed from agar slants. Horses so treated evinced marked symptoms, shortly after treatment, consisting of profuse sweating, uneasiness, increased repiration and exhaustion. In one instance death resulted. In most cases, however, the symptoms subsided in from thirty minutes to four hours after injection, with a noticeable increase in tolerance from day to day.

It is evident from these observations that the occurrence of *B. coli* as isolated from grain which was the causative factor of so-called "forage poisoning" bears no primary relation to the disease resulting from the feeding of the oats, but from a sanitary standpoint it seems advisable to protect animal feeds from *B. coli* contamination in so far as possible.

[2] Journal American Medical Association, 1901.

Further Studies of the Presence of and Significance of Agglutinins for Bact. abortus (Bang) in Cows' Milk. L. H. COOLEDGE.

An application has been made of the complement fixation and agglutination tests using *B. abortus* (Bang) as antigen and replacing the blood serum usually tested with milk. The two tests, when applied to milk from infected udders have checked closely, with the agglutination test a trifle more delicate and reliable. For this reason only the agglutination test is reported in this work.

In every instance where milk direct from the udder was found by animal inoculation or cultural methods to contain *B. abortus* it was also found to agglutinate *B. abortus*. Antibodies were apparently produced locally due to a local *B. abortus* injection as in some instances the milk from only one quarter would be positive while in others all four might be positive with a negative blood reaction. In other instances milk from a quarter would agglutinate the organism when the bacterium could not be demonstrated in the milk by animal inoculation. In these instances the agglutinins may have come from the blood but the indications are that they were produced locally by too slight an infection for the organism to be present in sufficient numbers to cause the disease with the 5 cc. of milk used for inoculation of guinea pigs.

The antibodies usually considered as accompanying infection by this organism have recently been found in the blood of two men and one woman drinking milk from a herd containing infected animals. In two other instances these antibodies appeared in the blood of men drinking milk that was known to be naturally infected with this organism. This method may prove to be another means of safeguarding certified and unpasteurized milk.

This material has been submitted to the *Journal of Agricultural Research.*

The Behavior of Streptococci of Human and Bovine Origin in the Cow's Udder.[1] GEORGE MATHERS.

Bacteriological observations in many epidemics of acute tonsillitis indicate that the causative organism is a virulent hemolytic streptococcus and that the infection is milk-borne. In epidemics in which an infected milk supply is an important factor it becomes necessary to determine the source of the bacteria, and the method by which they gain entrance into the milk. In the instance of epidemic tonsillitis the question naturally arises whether the udder of the suspected cow becomes infected with human streptococci, or whether the organisms causing the outbreak represent bovine streptococci that have suddenly acquired a heightened virulence for man. From a review of the literature it seems probable that hemolytic streptococci derived from bovine sources are of little sanitary significance, and the active factors in

epidemic sore throat are virulent streptococci of human origin. There is still some difference of opinion, however, as to the virulence of these human types of streptococci for the cow. Davis and Capps[2] have reported experiments in which they were able to produce mastitis in cows by the injection of hemolytic streptococci of human origin into the udder, and they have demonstrated conclusively that mastitis may exist in a cow's udder without any physical signs being present other than the invading bacteria and an increased number of leukocytes in the milk. Smith and Brown[3] are inclined to believe from their studies that the streptococci commonly associated with bovine mastitis are different from those found in epidemic sore throat and do not cause human throat infections. Moreover they infer that organisms of human origin do not cause bovine mastitis but may grow and multiply in the milk ducts, a condition which might explain outbreaks of tonsillitis. During the past year an experimental study[4] has been made of the comparative virulence of human and bovine types of streptococci for the cow, along with observations as to the behavior of these organisms over long periods of time in the cow's udder and the following results have been obtained.

In six instances mastitis has been produced in normal milch cows by the injections of small amounts of streptococcus cultures into the milk ducts. Streptococci derived both from human and bovine sources were used in these experiments. It was found that hemolytic streptococci with all the characteristics of the human type may be highly virulent for cows when injected into the milk ducts. They produce a severe mastitis which may result in an atrophy of the mammary gland. It was also observed that organisms of this type may grow and multiply in the milk ducts without causing any visible changes in the udder, but the milk in this instance contained an increased number of leukocytes and streptococci. Hemolytic streptococci from milk and the *Streptococcus lacticus* may produce an acute inflammation of the milk ducts but this change in my experience was of a transitory nature and the mammary gland regained its normal function very rapidly. In these observations streptococci derived from human sources proved to be more virulent for the cow than the milk strains.

In three instances of bovine mastitis all of which were due to hemolytic streptococci of the human type, there were no noteworthy changes in the morphology or cultural characteristics of the invading organisms observed in frequent examinations of the milk throughout the course of the infections. The distinguishing characters primarily noted for each organism are still present, and there are no modifications which might be considered as indicating a change from one type to another. These infections are still active 304, 272 and 234 days respectively after the udders were injected. Also there were no noteworthy changes

[2] Jour. Infec. Dis., 1914, xv, 135.
[3] Jour. Med. Res., 1915, xxxi, 455.
[4] Mathers, G., Jour. Inf. Dis., 1916.

in the distinguishing characteristics of the streptococci of the bovine type during the course of the corresponding experimental udder infections.

It is interesting to note that under experimental conditions the quarters of a cow's udder are apparently separate as regards infection, hence an examination of the milk from each quarter of the udder is necessary before a mastitis can be excluded in the case of a suspected cow.

Bacterial Changes in Uniced Specimens of Water. HENRY ALBERT, JACK J. HINMAN, JR., AND GHARRETT JORDAN.

It is well-known that bacteria tend to multiply rather rapidly in water allowed to remain at ordinary room temperature. The purpose of this investigation was to determine to what extent reliance may be placed on bacteriological examinations of water sent to a distant laboratory.

Examinations of forty different specimens of water of various degrees of purity, were made immediately after collection and again at the end of 8, 24, 48, and 72 hours. The standard methods of the A. P. H. A. were followed.

The following conclusions regarding the bacteriological findings are based on data obtained by a sanitary survey and by both chemical and bacteriological examinations:

1. The usual limit of 100 per cubic centimeter as the total number of bacteria on standard agar plates at 20°C. may fairly apply to uniced samples of water if examined within 8 hours after collection..

2. When uniced specimens of water are not examined until 24 hours after collection, the total number of bacteria at 20°C. which may be permitted in "safe" water may be placed at 200 per cubic centimeter and if not examined until 48 to 72 hours after collection, at 500 per cubic centimeter.

3. The presence of as many as 50 bacteria per cubic centimeter on standard litmus lactose agar at 37°C. should throw suspicion on water examined within 8 hours after collection although a total of 100 may be permitted if not examined until 48 hours after collection.

4. The presence of bacteria producing both acid colonies on standard litmus lactose plates and gas in standard broth throws suspicion on the water as polluted with sewage material, regardless of the length of time that the water has stood after collection.

5. The total number of bacteria in specimens of water which were polluted with sewage material (or probably so) as determined by both a sanitary survey and a chemical analysis is so high that it is not safe to establish limits of bacterial counts.

6. It is possible to depend on the results of bacteriological examinations of uniced specimens of water in a large proportion of cases provided the results are properly interpreted in the light of the sanitary survey, the chemical findings and the bacterial changes that occur in such specimens of water.

Further Studies on the Influence of a Lactose-Containing Diet upon the Intestinal Flora. Thomas G. Hull and Leo F. Rettger.

An ordinary bread and lettuce diet to which is added a considerable amount of lactose will simplify the intestinal flora of the white rat to a single group of organisms—the aciduric group. Milk has the same effect but to a less degree. In rats that have been kept on a high protein diet, *B. Welchii* and *B. coli* are prominent. When lactose is added to this diet the process is much the same as before but slower. All of the Welch bacilli and most of the colon bacilli disappear within five to ten days. The addition of meat to the lactose diet has very little effect if the aciduric flora has been previously established. Milk has but a slight effect upon the meat flora, probably due to the small amount of lactose present.

Three to four hours after feeding a meal containing dry lactose, sugar can be found in suspension for the entire length of the intestine, as well as in the feces. If the lactose is in solution when it is fed, it can be found as far as the ileum. Thus it is seen how lactose, being slowly absorbed, favors the multiplication of the aciduric group.

The reaction of the intestine apparently has little effect upon the flora, the acidity being no greater with the simplified flora than with the mixed flora.

Feeding Experiments with Bacterium pullorum. The Toxicity of Infected Eggs. Leo F. Rettger, Thomas G. Hull and William S. Sturges, Yale University.

The problem of eradicating ovarian infection in the domestic fowl must needs assume still greater importance than heretofore, in the light of recently acquired data. Not only is it of the greatest significance to eliminate the permanent carriers of *B. pullorum* from all flocks of fowls from the standpoint of successful poultry breeding, but also because they constitute a possible source of danger to man.

Eggs which harbor *B. pullorum* in the yolk in large numbers may produce abnormal conditions, when fed, not only in young chicks, but in adult fowls, young rabbits, guinea pigs and kittens. The "toxicity" for young rabbits is most pronounced, the infection usually resulting in the death of the animals. In kittens the most prominent symptoms are those of severe food poisoning with members of the para-typhoid group of bacteria. The possibility of infected eggs causing serious disturbances in young children and in the sick and convalescent of all ages must therefore receive due consideration.

Ovarian infection of fowls is very common throughout this country. Hence a large porportion of the marketed eggs must be infected with *B. pullorum*. The latter conclusion is warranted by the fact that of more than 13,000 fowls which were tested by the agglutination method fully ten per cent were positive, and therefore gave unmistakable evidence of infection with the organism in question. When eggs which harbor *B. pullorum* are allowed to remain in nests under broody hens, or in warm storage places, for comparatively few hours, they contain large numbers of the organisms.

Soft-boiling, coddling, and frying on one side only do not necessarily render the yolks free from viable bacteria; therefore, eggs which have gone through such processes may, like raw eggs, be the cause of most serious disturbances at least in persons who are particularly susceptible to such influence, and especially infants.

Studies in Bacterial Nutrition. The Utilization of Proteid and Non-Proteid Nitrogen. LEO F. RETTGER, WILLIAM S. STURGES AND NATHAN BERMAN, Yale Universty.

In a recent publication by Sperry and Rettger it was shown that bacteria are unable to utilize protein nitrogen without the preliminary cleavage of the proteins by enzymes, etc., into their relatively simple products. Further investigations clearly demonstrate that not only unheated (unchanged) proteins resist direct bacterial action, but that purified albumin which has been heated to the point of complete coagulation and sterilization likewise remains unaffected.

It also appears quite certain that albumoses and peptones are not attacked by bacteria, or at the most but feebly, without the aid of a proteolytic enzyme, strong acids, alkali, or extreme heat. Organisms like *B. coli* and *B. typhi* which do not elaborate proteolytic enzymes are unable, therefore, to make free use of albumose and peptone nitrogen. This has been shown in culture tests with weak solutions of both the untreated and partially purified Witte's peptone. For the determination of any possible loss of proteose and peptone, or of albumin, as the case may be, the quantitative biuret method as used and recommended by Vernon has been employed with considerable satisfaction.

What is often regarded as autolysis of *B. coli* and other gelatin-non-liquefying bacteria is not a process of digestion of the protein constituents of the bacterial cells, since there is no reduction in the amount of protein of the medium plus the suspension, and if the protein partially disappears from the cells it is due to agencies other than enzymes, as for example small amounts of acid or alkali, and perhaps mere washing.

A proteose or peptone-digesting enzyme, erepsin, has not been demonstrated in any of the experiments.

Yeasts, Probably Pathogenic, Recovered from Routine Throat Cultures. ARTHUR L. GROVER.

In the past various observers have noted the presence of yeast-like bodies in smears from the throat but no real attempt has been made to study these.

The present investigation covers ninety-cultures showing yeast-like bodies. Fifty-six gave yeast cultures, 3 oidia, 2 leptothrix, 20 gave molds, and 9 gave no fungus. It is interesting to note that the molds and yeasts have identical morphology in the primary smears.

These 56 yeasts could be divided into 17 distinct varieties as shown by the following table:

No.	DEXTRIN	GLUCOSE	GALACTOSE	LACTOSE	LEVULOSE	MALTOSE	MANNIT	RAFFINOSE	SACCHAROSE	INDOL	INULIN	GLYCERIN	TOP YEAST	APPEARANCE ON SLANT AGAR
1	—	gas*	*	*	*	—	*	*	—	*	—	—	—	Yellowish green heaped up, confluent
2	—	*	—	—	—	—	—	—	—	*	—	*	*	Thin whitsh film
3	—	*	*	*	—	—	—	—	—	—	—	—	*	Yellowish green, flat, confluent
4	—	gas*	—	—	gas*	—	—	—	*	—	—	*	*	Bright lemon yellow, heaped up, confluent
5	*	gas*	*	—	—	—	*	*	*	—	—	—	—	At first white, later pink, confluent
6	gas*	gas*	gas*	gas*	gas*	gas*	gas*	—	gas*	—	—	*	*	Like sheets of yellow paint
7	—	*	*	—	*	gas*	*	—	*	*	—	*	—	Waxy white, confluent, raised up
8	gas*	gas*	gas*	gas*	gas*	gas*	gas*	gas*	gas*	gas*	—	*	*	Creamy white, rather flat and dry
9	—	*	*	—	*	*	—	—	—	—	—	*	*	Golden yellow, like sheets of paint
10	—	*	gas*	—	—	—	—	—	gas*	—	—	—	—	Dirty gray, dry film
11	*	—	*	—	—	—	—	—	—	—	—	—	*	White like mass of cream cheese
12	*	*	*	—	*	*	—	—	*	—	—	*	*	Greenish yellow, heaped up
13	*	gas*	*	*	gas*	*	—	—	*	*	—	—	—	At first white, then yellowish, finally fawn colored, confluent
14	—	*	—	—	—	—	—	—	*	—	—	—	*	Salmon pink, confluent
15	—	gas*	*	*	gas*	—	—	—	—	N*	—	—	—	White discrete colonies hirsute
16	*	*	—	—	*	—	—	—	—	*	—	—	*	Gray, thin film.
17	*	*	*	—	*	*	—	*	*	—	—	*	*	Pale yellow discrete colonies

* = Acid or top yeast.
N = Nitrites

Subcutaneous injection into guinea pigs in eleven cases gave a general glandular enlargement. It was possible to recover the yeasts from the glands. Eight of these gave a false membrane in guinea pigs when rubbed on an abraded surface of the mucous membrane lining the cheek. In three cases this membrane extended down over the entire throat. The clinical history of the cases from which the yeasts were recovered in numerous cases showed membranous angina and the absence of the *Bacillus diphtheriae.*

ABSTRACTS OF AMERICAN BACTERIOLOGICAL LITERATURE

BACTERIOLOGY OF FOOD

Effects of Refrigeration Upon the Larvae of Trichinella spiralis. B. H. RANSOM. (J. Agr. Res., 1916, *5*, 819–854).

This work was planned to show whether the refrigeration of meat was a safeguard against the spread of trichinosis. Trichinous meat was kept for periods varying from a few minutes up to fifty-seven days at various temperatures below the freezing point of water, and then after gradual thawing was fed to test animals, generally rats. Refrigeration at temperatures as low as 50°C. for twenty days or longer, although not always killing the larvae, so influenced them that the meat could no longer cause infection. A temperature of 41°C. generally killed them in ten days or less. The author concludes that a refrigeration for twenty days at 41°C. may be regarded as always sufficient to render trichinous meat safe for consumption.—H. J. C.

The Bacterial Examination of Sausages and Its Sanitary Significance. W. E. CARY. (Amer. Jour. of Public Health, 1916, *6*, 124–135).

The author found that the bacterial content of sausages bears no relation to the sanitary conditions of the shop. The average count of 16 samples taken from shops scored by the author as insanitary was 24,000 per gram at 37°C. and 2,133,000 at 20°C., while the count of 18 samples collected from sanitary shops was 241,000 per gram at 37°C. and 13,280,000 at 20°C. *B. coli* was found in 94 per cent of the samples. Organisms biologically related to, but not identical with, the enteritidis group were present in 25 per cent of the samples, and *Proteus vulgaris* was found in 33 per cent of them. Starch as an adulterant was detected in 56 per cent of the samples. Skins used as casings, if properly prepared, cannot be considered to increase the bacterial content. Cooking destroyed from 93.3 per cent to 100 per cent of the bacteria present.—D. G.

BACTERIOLOGY OF SOILS

Some Factors Influencing the Longevity of Soil Micro-organisms Subjected to Desiccation, with Special Reference to Soil Solution. WARD GILTNER AND H. VIRGINIA LANGWORTHY. (J. Agr. Res., 1916, *5*, 927–942.)

It has been observed in the past that bacteria are able to resist drying for longer periods in soil than under other conditions. This has been thought to be due to the retention by the soil of moisture in hygroscopic

form. This cannot be the only factor, however, for the longevity of bacteria in various soils is not proportional to the grain-size and hygroscopic moisture.

Recently Van Suchtelen has succeeded in extracting the soil-solution directly from soil. It was found in the course of the present experiments that if bacteria are suspended in the solution extracted by Van Suchtelen's method from a rich clay loam and are then mixed with sand and dried, they live longer than if suspended in physiological salt solution and then dried under similar conditions. This suggests that the reason why bacteria resist drying longer in a rich clay loam than in sand is not only because of the greater amount of hygroscopic moisture present but because there is something present in the soil-solution of the loam that has a protective influence upon the bacteria. The soil-solution was found by Van Suchtelen to contain a slimy material; and the writers suggest that this might be the substance protecting the bacteria when dried.—H. J. C.

A Comparison of the Acid Production of the B. coli Group Isolated from Various Sources. W. W. BROWNE (Amer. Jour. of Public Health, 1916, *6*, 39–48).

The author undertook this study to determine the amount of acid production in various carbohydrate solutions by members of the *B. coli* group, as a guide to the recentness or remoteness of pollution of oysters in Narragansett Bay. He found that members of this group isolated from either feces or oysters produced their maximum amount of acid in lactose and glucose when incubated at 37°C. for 24 hours; furthermore that the maximum amount of acid was produced by the end of 24 hours. One series of experiments showed that the largest amount of acid was produced in the monosaccharides and hexites (glucose, levulose, galactose, arabinose, xylose, isodulcite, mannite), less in the disaccharides (lactose, maltose), and least in the trisaccharide (raffinose). That is to say the yield of acid varies inversely as the complexity of the sugar. The author concludes that the members of the *B. coli* group isolated from feces produce more acid in carbohydrate solutions than cultures isolated from oysters, the average differences being very slight, but apparently consistent in all the different fermentable media studied.—D. G.

Relation Between Certain Bacterial Activities in Soils and Their Crop-Producing Power. PERCY EDGAR BROWNE. Journal of Agricultural Research 1916, *5*, 855–869.

These experiments as a whole represent a line of investigation in soil bacteriology which it is believed will ultimately place the subject on a more practical basis—a basis which will permit the direct application of the results obtained to the solution of soil-fertility problems. The relations between the bacterial activities studied and the actual crop yields on these plots have proved so striking and so consistent that it was felt that accidental coincidence had been practically elimi-

nated and the results might be considerd to give a strong indication that certain bacterial activities in fields are very closely associated with crop yields. Furthermore, the tentative conclusion presents itself that tests of such bacterial activities in the laboratory may indicate quite accurately the crop-producing power of a soil, or, at least, the relative crop-producing power of several soils. If, further, more exhaustive tests confirm these preliminary observations, it may be possible to secure advance information regarding the crop-producing power of soils by means of laboratory tests of bacterial action in those soils.— S. H. A.

BACTERIOLOGY OF WATER AND SEWAGE

Predicts Federal Control of Stream Pollution. EARLE B. PHELPS. Eng. Record, 1916, *73*, 173–4.

Federal policy needed in the supervision of stream conditions and the necessary administrative body to put it into effect.—F. B.

Air Diffusers Tested at Milwaukee, (Wis.) Sewage Plant. T. CHALKLEY HATTON. Eng. Record, 1916, *73*, 255. Ill. Sec. Amer. W. W. Assn. 1916.

Filtros plate, composed of quartz sand baked, of uniform porosity has given the most satisfactory results. The removal of 90 per cent of suspended matter, 95 per cent bacteria and an effluent stable for 5 days was secured at Milwaukee with the continuous flow tank by using 1.75 cubic feet of air per gallon sewage with 4 hours aeration, 20 per cent activated sludge and from 10 to 15 minute sedimentation. The estimated cost is $4.38 per million gallon excluding engine room and plant attendance, and the cost of disposing of the sludge.—F. B.

DAIRY BACTERIOLOGY

Fermented Milks. L. A. ROGERS. Bulletin 319, U. S. Dept. Agr.

A brief résumé of our present knowledge of this subject. The therapeutic and food value of fermented milk is discussed together with the method of preparation of buttermilk, kefir and yogurt. S. H. A.

The Present Status of the Pasteurization of Milk. S. HENRY AYERS. Bulletin 342. U. S. Dept. Agr.

A summary of our present knowledge of the process of pasteurization. The subjects discussed are: Meaning of the term pasteurization; value of pasteurization; extent of pasteurization in the United States; methods of pasteurization; advantages of low temperature pasteurization; temperatures and methods most suitable for pasteurization; supervision of the process of pasteurization; handling pasteurized milk; cost of pasteurizing milk; bacteria which survive pasteurization; modern theories of pasteurization; and the necessity for pasteurization. —S. H. A.

Agglutination Test as a Means of Studying the Presence of Bacterium abortus in Milk. L. H. COOLEDGE. (J. Agr. Res., 1916, *5*, 871–875).

In testing a large number of samples of milk to determine the presence of the causal organism of contagious abortion, the only previously proposed technique that proved available was animal inoculation—an unsatisfactory procedure because of the length of time required. The writer, has therefore, worked out a method of employing the agglutination test, using 48-hour agar cultures of *B. abortus* as antigen. Negative results by this test always indicated absence of the organism in question; but positive results did not necessarily prove its presence. In making a long series of tests, however, it was found to reduce the number of suspicious cases sufficiently so that the use of animal inoculation was practical in those few cases in which some particular cow's milk did cause agglutination.—H. J. C.

Study of Condensed and Evaporated Milks. IDA A. BENGSTON. Jour. Home Econ., 1916, *8*, 29–33.

The present extensive use of condensed and evaporated milk products makes the proper control of manufacture, and the establishment of standards of purity and food value imperative. Meager work has been done on the bacteriology of these milk products. The methods of preparation of evaporated milk may assure a sterile product. This is not true, however, of condensed milk. The bacteria found are chiefly those that survive pasteurization, and their number may be as high as 1,000,000 per cc. The high sugar content of the condensed milk inhibits the multiplication of many forms.

Streptococci, staphylococci, *B. sporogenes*, lactic acid producing bacilli, *B. subtilis, B. mesentericus, B. coli*, and yeasts have been found. —C. M. H.

DISINFECTION

Phenol Coefficient of Germicides. F. B. KILMER, A. W. CLARK AND P. HAMPTON. (Jour. Ind. and Eng. Chem., 1916, *8*, 45.

Study of reliability of Hygienic Laboratory method for testing disinfectants. Tests made in two laboratories gave concordant results provided following medium was used: Liebig's extract, 3 grams; salt, 5 grams; Peptone (Witte), 10 grams; water, 1000 cc.; Composition of medium important.—I. J. K.

IMMUNOLOGY

Anaphylatoxin and the Mechanism of Anaphylaxis. RICHARD WEIL. Proc. Soc. Exp. Biol. and Med., 1915, *13*, 37–39.

Precipitin is identical with the antibody effective in passive sensitization. Precipitating antibody heated at 72° for one-half hour lost its capacity to bind complement in the presence of antigen but still retained its sensitizing value as shown by injection into animals. The

conclusion is drawn that complement plays no part in the anaphylactic reaction and therefore that anaphylatoxin plays no rôle in this phenomenon.—W. J. M.

On the Mechanism of Anaphylaxis and Antianaphylaxis. J. BRONFEN-BRENNER. Proc. Soc. Exp. Biol. and Med., 1915, *13*, 19–21.

The author regards anaphylaxis as due to toxic split products of the normal serum proteins produced by the action of the normal tryptic ferment of the blood after the inhibitory influence of the colloids has been diminished by the specific interaction of antigen and antibody. Antianaphylaxis is explained as the result of antitryptic influence of split products of products of proteolysis.—W. J. M.

Agglutination of Bacteria in vivo; Its Relation to the Destruction of Bacteria Within the Infected Host and to Septicaemia. C. F. BULL. Proc. Soc. Biol., and Med., 1915, *13*, 32–33.

Intravenous injection of immune serum causes an abrupt clumping of bacteria in the circulating blood in bacteremia and their accumulation in the internal organs, where they are phagocyted.—W. J. M.

The Utilization of "Reactor" Milk in Tuberculo-medicine. C. B. FITZPATRICK. Proc. Soc. Biol. and Med., 1915, *13*, 35–37.

Cows in excellent physical condition, but reacting to tuberculin, were used. Seven patients with moderately advanced pulmonary tuberculosis were fed upon their milk and showed improvement as compared with control cases on normal milk.—W. J. M.

Late Results in Active Immunization with Diphtheria Toxin-Antitoxin and with Toxin-Antitoxin Combined with Diphtheria Bacilli. W. H. PARK AND ABRAHAM ZINGHER. Proc. N. Y. Path. Soc., 1915, N. S. *15*, 110–116.

Individuals giving a negative Schick test before treatment are probably immune for life. Those who give a positive Schick test and who have been exposed to diphtheria should receive either antitoxin alone or, for longer protection, both antitoxin and toxin-antitoxin injections. For general prophylaxis against diphtheria, not including immediate contacts, toxin-antitoxin alone, or with the addition of killed diphtheria bacilli, is recommended. The dose is 1 cc. of toxin-antitoxin (85 to 90 per cent of the L+ dose of toxin to each unit of antitoxin) and 1,000,-000,000 bacteria, injected subcutaneously three times at intervals of six or seven days. The early and the late results should be controlled by the Schick test, early results within four weeks and late results from four months to two years after the immunizing injection.—W. J. M.

Agglutination in Pertussis. O. R. POVITZKY AND E. WORTH. Arch. Int. Med. 1916, *17*, 279–292.

The Bordet-Gengou bacillus, grown upon coagulated horse blood veal agar, is readily agglutinated by immune serum. An agglutinating

serum can generally be obtained from rabbits after ten or twelve intra-peritoneal injections of living bacilli at seven day intervals. The agglutination test demonstrates the unity of the pertussis group and differentiates this group from the hemoglobinophilic and pertussis-like organisms. In the diagnosis of pertussis a positive agglutination test at a dilution of 1: 200 is necessary, in order to eliminate the presence of natural agglutinins.—G. H. R.

Treatment of Typhoid Fever by Intravenous Injections of Polyvalent Sensitized Typhoid Vaccine Sediment. Studies in Typhoid Immunization VI. F. P. GAY AND H. T. CHICKERING. Arch. Int. Med. 1916, *17*, 303–328.

The report deals with the treatment of 53 cases of typhoid fever, the diagnosis being confirmed by laboratory examination, with the sensitized vaccine of Gay and Claypole. The treatment consists of one or more intravenous injections of 1/50 to 1/25 milligram of the vaccine, or, in some cases, an intravenous injection followed by three subcutaneous injections of 1/10 milligram each. The symptoms following the intravenous injections are mild, and the results generally beneficial unless the dose is too large. Of these 53 cases, 66 per cent showed distinct improvement and 34 per cent were relatively unaffected. The curative results are regarded as due to the hyperleukocytosis and the increased amount of antibodies induced by the vaccine. In a few patients having low antibody (agglutinin) titer the vaccine treatment was supplemented by the intravenous injection of considerable amounts of typhoid-immune goat serum. The superiority of sensitized over non-sensitized vaccine is due to the production of a specific hyperleukocytosis.—G. H. R.

The Mechanism of the Abderhalden Reaction with Bacterial Substrates. G. H. SMITH AND M. W. COOK. Jour. Infect. Diseases 1916, *18*, 14–19.

Bronfenbrenner, working with tissue substrates had arrived at the conclusion that the Abderhalden reaction can be resolved into two distinct sub-phases; (1) sensitization of the substrate by specific elements of the immune serum, resulting in adsorption of antiferments; (2) autodigestion of the serum; he also concluded that only the former of these reactions was specific. The present authors attempt to ascertain whether the same principles apply to the reaction when bacterial instead of tissue substrates are employed. Immune sera were obtained from rabbits immunized to (a) typhoid, (b) paratyphoid A, (c) *Staphylococcus aureus*; also serum from control rabbits. The serum of each rabbit was combined with its homologous substrate and also with the two non-specific substrates. After the serum-substrate contacts, the tubes were centrifuged and the sera dialyzed, and tested by the Ninhydrin method. The substrates were washed and each divided into four parts, to three of which fresh serum from the immunized rabbits was added; the fourth received normal serum. Contact in cold was allowed for 16 hours, after which the tubes were centrifuged,

the serum dialyzed and tested. In the first place, each serum after having been combined with its specific bacterial substrate, reacted positively, the other combinations being negative. In the second place, each substrate that had already been so combined with its specific serum, upon being subsequently combined with the non-specific sera, acted on all of them so as to yield a positive reaction upon dialysis, thus demonstrating that this phase of the reaction is due to autodigestion of the serum and is non-specific. Whether the sensitization of the substrate corresponds with the usual antigen-antibody reaction is a point left for further study.—P. B. H.

LABORATORY TECHNIQUE

On a Colorimetric Method of Adjusting Bacteriological Culture Media to any Optimum Hydrogen ion Concentration. S. H. Hurwitz, K. F. Meyer and Z. Ostenberg. Proc. Soc. Exp. Biol. and Med., 1915, *13*, 24–26.

The indicator is phenolsulphonephthalein 0.01 per cent. The final adjustment is made after sterilization of the medium, with aseptic technic, the readings being made in a specially devised comparator against a standard color solution.—W. J. M.

The Use of Brilliant Green for the Isolation of Typhoid and Paratyphoid Bacilli from Feces. Charles Krumwiede, Jr., Josephine S. Pratt and Helen I. McWilliams. Jour. Infect. Diseases, 1916, *18*, 1–13.

The success of the authors and others in the use of brilliant green broth for the enrichment of typhoid and paratyphoid bacilli in feces led to the attempt to produce a dye agar. After many trials a medium of the following constitution was found to be satisfactory. Extract of beef (Liebig's) 3 gm., Witte's peptone 10 gm., salt 5 gm., agar 15 gm., water 1000 cc. Dissolve in autoclave; the final reaction is set to the Andrade indicator, adding 1 cc. to a 100 cc. bottle of agar; the reaction may be set at time of preparation or (preferably) when used. If the latter, after dissolving, render slightly alkaline to litmus, bottle in 100 cc. amounts and autoclave. Just before use, adjust 0.6 to 0.7 per cent to phenolphthalein (hot titration) then add to each 100 cc. 1 per cent lactose and 0.1 of glucose (25 per cent sterile solutions) and finally the appropriate amount (0.2, 0.3 or 0.4 cc.) of a 0.1 per cent solution of brilliant green. Use about 16 cc. of agar for each plate, allowing them to stand open until agar has cooled. Inoculate as in Endo plates. The method of use is as follows: Rub up in extract broth a large sample of feces (1:15 by volume.) Place one loop of suspension on a 0.2 cc. and on a 0.3 cc. plate; streak in order given and then on an Endo plate. Place two loops on each of a similar pair of green dye plates; streak in same order and then on Endo plate. Use a heavy platinum wire looped at end. For a direct agglutination test a macroscopic slide method is employed. For fishing, the Russell medium, with 1 per cent Andrade indicator substituted for litmus is employed. As an added precaution

it is recommended that there be inoculated from the original fecal suspension 0.1 cc. into 1 per cent glucose extract broth containing 1 : 300,000 of the brilliant green. If slight growth develops on the green plates, Endo agar is inoculated from the broth tubes after 18 hours. In tests made upon carrier and normal stools, and of convalescents prior to discharge it was found that many fecal types were restrained while the typhoid bacilli developed well. In one instance the positive results were increased 36 per cent over Endo plates. The method also proved successful for the isolation of members of the paratyphoid-enteritidis group from feces.—P. B. H.

MEDICAL BACTERIOLOGY

The Effect of Continuous Electric Light in Experimental Arthritis. W. E. SIMMONDS AND J. L. MOORE. Arch. Int. Med. 1916, *17*, 78–81.

Exposure to continuous incandescent electric light prevented or rendered less severe experimental streptococcal arthritis in rabbits. When the light treatment was begun after the development of arthritis, treated animals improved, while control animals continued to develop new lesions.—G. H. R.

Lesions Produced in Rabbits by Repeated Intravenous Injections of Living Colon Bacilli. C. H. BAILEY. Proc. Soc. Exp. Biol. and Med., 1915, *13*, 62–63.

Colon bacilli were injected intravenously into rabbits at 3–4 day intervals over long periods. Animals surviving 88 to 142 days showed fibrous and hyaline changes in the kidneys, spleen and liver. In the spleen a material resembling amyloid was formed about the Malpighian bodies but the amyloid nature of this substance was not conclusively demonstrated.—W. J. M.

Tuberculosis in Infancy. C. H. DUNN, Amer. Jour. Diseases of Children, 1916, *11*, 85–94.

The author briefly reviews the various opinions that have been held concerning the portal of entry and the type of the organism in tuberculosis of children. The observations recorded consist of twenty-five autopsies upon infants under two years of age. The examinations were particularly directed toward the lungs and intestines, which were cut into small pieces and all suspicious portions sectioned and examined microscopically. In twenty-two of the twenty-five cases there was found what was regarded as the primary focus and portal of entry. The author therefore, disagrees with the opinion that the tubercle bacillus may in many cases enter the body and leave no local histological evidence. In twenty of the cases the supposed primary was located in the lung and in two it was found in the intestine. In only five cases were animal inoculations made and the type of organism studied. Four of these proved to be human and one bovine. The one bovine culture came from one of the cases in which the primary lesion was located in the intestine.—R. M. T.

The Bacterial Flora of Infected Gun Shot Wounds.—LOUIS A. LaGARDE. The Military Surgeon—1916, *38*, 1–6.

This article is written for the benefit of the military surgeon rather than for the bacteriologist, but reviews some of the bacteriological work that has been done on wounds in the present war. Thus Fleming examined 127 wounds and found that the *B. Welchii* was present in 103, *Bacillus tetanus* in 22, and streptococci in 102 during the first week. Gudgeon, Gardner, and Bawtree found that of 100 wounds all were infected, 99 with various combinations of aerobic and anaerobic bacteria, and one with a pure culture of *B. Welchii*. The article points out that with regard to the bacteriology of gun shot wounds, investigation during the present world war has so far resulted in no new bacteriological data.—E. B. V.

Practical Points in the Prevention of Asiatic Cholera. ALLAN J. Mc-LAUGHLIN, The Military Surgeon, 1916, *38*, 22–29.

McLaughlin quotes literature showing that presumably healthy individuals have been proven to harbor cholera vibrios in dejecta for periods ranging from 10 days to 69 days, and that Gaffky reported a case who was a carrier for 6 months. The carrier question has therefore become one of the most important factors in any endeavor to stamp out cholera or prevent the entrance of the disease. These long time carriers make a farce of the ordinary 5 day quarantine detention without stool examination. Instead of this, the present method is to examine the stools of all contacts or suspects. In view of the fact that prompt diagnosis is essential, and to avoid time consuming manipulations where large numbers of people are to be examined, the following simple method is recommended: Plate on agar after primary inoculation in peptone enriching media, and test individual suspicious colonies by a macroscopic agglutination on a glass slide, using a very powerful cholera immune serum, which will agglutinate cholera in dilution of 1–4000. This serum may be used in dilution of 1–200 and in this strength will give prompt agglutination with cholera but not with other organisms. Goldberger's enriching solutions, an alkaline egg peptone and on alkaline meat infusion peptone are mentioned with the statement that laboratory tests indicate that they restrain the growth of ordinary faecal bacteria while promoting the growth of cholera vibrios, but that these media have not yet been tested in actual field work.—E. B. V.

PHYSIOLOGY OF BACTERIA

Effect of Natural Low Temperature on Certain Fungi and Bacteria. H. E. BARTRAM. (J. Agr. Res., 1916, *5*, 651–655.)

Dried cultures of certain molds, Actinomycetes, and bacteria proper were exposed to outdoor conditions at temperatures sometimes as low as −30°C. More than half of the molds survived for four months under these conditions, but most of the bacteria died. Control cul-

tures in the laboratory did not die. Writer does not state whether the cultures kept outdoors were exposed to sunlight as well as to cold.—H. J. C.

Effect of Elemental Sulphur and of Calcium Sulphate on Certain of the Higher and Lower Forms of Plant Life. WALTER PITZ. (J. Agr. Res., 1916, *5*, 771–780.)

These experiments were planned because there has been some disagreement in the past as to whether sulphur compounds increase or decrease plant growth. Tests were made to observe the effect of elemental sulphur and of calcium sulphate upon: (1) total number of bacteria in soil (determined by plate method), (2) growth of pure cultures of the organism causing red clover nodules, (3) accumulation of nitrates and ammonia in soil, (4) growth of clover in soil and in agar culture. The results indicate that elemental sulphur slightly stimulates the growth of red clover, but has a harmful effect upon all the other activities investigated; that calcium sulphate increases the growth of the legume organism and the growth of clover, but has no influence upon the general soil bacterial flora.—H. J. C.

The Action of Schumann Rays on Living Organisms. W. T. BOVIE. Bot. Gaz., 1916, *61*, 1–29.

The source of light was a hydrogen discharge tube, the top of which was closed by a transparent fluorite plate through which the Schumann rays were emitted. In general a small organism was killed more quickly than a large one. The organisms used were rotifers, amoebae, infusoria, Spirogyra and fungus swarm spores. By a number of methods it was shown that the action of the light is on the organism directly and not indirectly by the formation of a toxic substance in the medium. The extreme destructive action of these rays is the result of strong absorption. Because of this absorption, the Schumann rays have a marked localized action, which gives them a peculiar value for investigations in the morphology and physiology of the cell. The change produced is often one which results in an alteration of the equilibrium of the water content of the protoplasm. In the Schumann region of the spectrum, as in the region of longer wave length, the destructive action of the light increases as the wave length decreases, and the light of the Schumann region is much more destructive than the light of the region of longer wave length.—J. T. E.

PLANT PATHOLOGY

A Serious Disease in Forest Nurseries Caused by Peridermium filamentosum. JAMES R. WEIR AND ERNEST E. HUBERT. Jour. Agr. Res., 1916, *5*, 781–785.

Peridermium filamentosum Peck has been found to cause a serious disease of yellow pine seedlings at the Savenac nursery located at Haugan, Mont. The fact that the same species of Peridermium at-

tacks both the lodgepole pine and the yellow pine increases the difficulty of control of this fungus.—S. H. A.

Sweet Potato Scurf. L. L. HARTER. Jour. Agr. Res., 1916, *5*, 787–793.

The scurf disease of the sweet potato was first recognized in 1890 by Halsted, who named the fungus *"Monilochaetes infuscans,"* a new genus and species. He failed, however, to describe either the genus or species. The scurf has been found prevalent in nine States and sparingly in others, and on 16 varieties of sweet potatoes. The organism has been shown by inoculation experiments to be the true cause of the disease. A detailed discussion of the morphology of the organism is taken up, also its growth on different culture media at different temperatures. It was found that the organism on the host consisted merely of sporophores and conidia. In the culture, however, well-defined branched mycelia and spores developed.—S. H. A.

Further Studies on Peanut Leafspot. FREDERICK A. WOLF. Jour. Agr. Res., 1916, *5*, 891–902.

A continuation of work on the fungus diseases of peanuts, the object being to secure information regarding the agencies concerned in the distribution of leafspot, *Cercospora personata* (B. and C.) Ellis, and to correlate the destructiveness of the disease with the presence of certain climatic conditions. Crop rotation was not found effective under field conditions in eliminating leafspot; nor was the disease prevented by seed disinfection with copper sulphate or formaldehyde before planting. No correlations between the presence of certain conditions of temperature and moisture and the presence of leafspot exist because of the fact that air currents and certain insects are carriers of *Cercospora personata.*—S. H. A.

Soil Stain, or Scurf of the Sweet Potato. L. J. TAUBENHAUS. Jour. Agr. Res., 1916, *5*, 995–1001.

The economic importance of the disease is discussed, also the occurrence of soil stain, symptoms of soil stain, effect of the disease on the host, factors favorable to soil stain development, the cause of soil stain or scurf and the morphology and physiology of the fungus causing the scurf. The fungus *Monilochaetes infuscans* was found to be difficult to culture because it is a very slow grower and is readily overrun by associated saprophytes. The conidiophores of *M. infuscans* are distinct from the mycelium, the older growth of which is also dark. The conidia are borne in chains which readily break up when moistened or disturbed.—S. H. A.

Factors Involved in the Growth and the Pycnidium Formation of Plenodomus fuscomaculans. GEORGE HERBERT COONS. Jour. Agr. Res. 1916, *5*, 713–770.

This paper gives the result of experiments performed with *Plenodomus fuscomaculans*, a fungus pathogenic to the apple. The organ-

ism was found to have a wider range of conditions suitable for growth than for reproduction. The quantity of food stuffs necessary for growth is extremely minute. Pycnidium production requires more food, but the meager amount present in distilled water is sufficient to allow the production of a few pycnidia. Magnesium sulphate and potassium dihydrogen phosphate in very dilute solutions furnish the necessary mineral elements for growth and reproduction. The carbon supply may be taken from a wide range of compounds of alcoholic structure. Carbohydrates furnish food material in the most available form, and of these xylose and maltose produce the best growth. The nitrogen assimulation is greatly influenced by the type of carbon nutrition. The influence of physical conditions on growth and reproduction is also shown. The general problem of the paper was to study the effect of environmental factors upon *Plenodomus fuscomaculans* especially as they influenced growth and reproduction.—S. H. A.

PRELIMINARY REPORT ON SYNTHETIC MEDIA[1]

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THEORETICAL DISCUSSION

By a "Synthetic Medium" is meant a solution which contains only compounds of known composition and structure. Any medium which includes compounds of unknown composition or structure is not a synthetic medium. This paper deals both with synthetic nutrient solutions, and, with solid synthetic media formed by the precipitation of an agglutinant from compounds of known composition and structure. In the latter case, the agglutinant formed by precipitation from known compounds should have a known structure and composition. We should properly exclude from the list of synthetic media all those which contain substances of unknown composition or structure, such as meat extracts, proteins of unknown structure, agar and gelatine. In order, however, to illustrate the present conception of possible media, the scheme presented includes many compounds of unknown composition and structure, such as the polysaccharids, tannins, glucosides and proteins. These compounds which have either an unknown composition or unknown structure are placed in their logical position in the systematic arrangement, because their decomposition products are more or less known and because in nature they, or their decomposition products, furnish the principal source of energy to saprophytic microorganisms, and because it may be necessary to fall back upon some of them in order to secure media for such microorganisms as cannot utilize media made of simpler compounds. However, media which contain such compounds of unknown composition or structure cannot properly be classed as synthetic.

It is clear from the results of numerous investigators that microorganisms can utilize various carbohydrates, alcohols and salts of fatty acids as sources of carbons, both for energy material and cell construction. It has, also, long been known that the optical isomers of a substance containing an asymmetrical carbon atom behave very differently toward biological agents, such as yeast, molds, and bacteria. The classical researches of Pasteur showed that *Penicillium glaucum*, assimilated only d-tartaric acid and not the l-tartaric acid. Recent investigations have shown, however, that this organism will also decompose the l-tartaric but less rapidly than the d-tartaric form. Likewise there are other organisms, bacteria, yeast and molds, some of which prefer a d-form of an isomeric compound while others prefer the l-form of the isomer. Investigations of the action of yeast on all the known hexoses has shown that only four are fermented, viz., the d-forms of glucose, mannose, galactose and fructose. When the behavior of different species of yeast toward these hexoses is studied, it is found without a single exception that any species of yeast which ferments any one of the three hexoses, glucose, mannose or fructose, likewise ferments all three of them. We know, too, from the work of E. Fischer and his associates that certain enzymes which are able to decompose certain organic compounds cannot attack their opposite isomers. Thus, for example, α-methyl glucoside is hydrolyzed by the maltase (α-glucosidase) of yeast, while β-methyl glucoside is hydrolyzed by emulsin (β-glucosidase). Emulsin does not act on the α-glucoside and maltase has no effect on the β-glucoside. So fundamental is this action that the composition of an organic compound may often be determined by its behavior in the presence of an enzyme. While lack of knowledge concerning the behavior of optically inactive compounds toward enzymes does not permit us to speak as definitely as we may concerning the optically active, nevertheless it is probable that an enyzme is capable of attacking only a certain group, or formation. Such, for example, is the action of myrosin upon sinigrin and other sulphur containing glucosides, where the change is confined to the sulphur linkage. This

property of the enzyme no doubt regulates the ability of the parent cell to utilize a compound, consequently, organisms can utilize as a source of energy and carbon for cell construction only those compounds whose configuration, or the configuration of a radical therein, matches the configuration of their enzymes. By commencing with the simplest carbon compounds capable of yielding energy we may, by admitting only one energy material at a time build up a series of synthetic media which are mutually inclusive and exclusive. That is, those organisms which have an enzyme in common can utilize a given compound, while those which do not possess the necessary enzyme will not develop, providing the compound is not too complex; otherwise, some of the decomposition products may have a suitable configuration. We find that carbon compounds having a certain configuration or certain atomic groups can be utilized by all those microorganisms which develop the requisite enzyme. Therefore, as far as energy is concerned, it is possible to prepare a medium which will allow the growth of only a given number of organisms.

Numerous investigators have shown that bacteria as well as molds can utilize ammonia nitrogen. Gerlach and Vogel isolated and studied several forms of bacteria which were capable of utilizing nitrate nitrogen. It is a well known fact that a large number of molds also readily assimilate nitrate nitrogen. Renato Perrotti pointed out the fact that certain bacteria were capable of utilizing cyanide nitrogen. The assimilation of either ammonia, nitrate or cyanide nitrogen was dependent, always, upon the presence of suitable energy materials.

Previous work indicates that the other nutrient elements necessary, for bacteria at least, are probably common to all, therefore, the development of synthetic media must follow two lines of cleavage—first, according to carbon compounds required for energy, and second, according to nitrogen compounds required for nutrition.

In order to make the résumé complete we need only mention those bacteria which require a source of carbon for energy and utilize free nitrogen, those which obtain their energy by the

oxidation of ammonia or nitrite nitrogen, those bacteria which obtain their energy by the oxidation of sulphur and those which obtain their energy from the oxidation of iron carbonate. We are now in a position to summarize the food requirements of microorganisms. They need in general the common nutrient salts. Their nitrogen requirements differ, some being able to utilize ammonia, some nitrate, some cyanide and probably some nitrite nitrogen. They differ too in the compounds which they use as sources of energy. Among the prototrophic forms we find, respectively, those groups which can utilize iron, sulphur, ammonia, nitrite or a non-nitrogenous organic compound as a source of energy, while among the saprophytic and parasitic forms we find those which can utilize the non-nitrogenous organic compounds as sources of energy, as well as those which can utilize nitrogenous organic compounds. If we prepare nutrient synthetic solutions containing one of the simplest nitrogen and one of the simplest carbon compounds there will grow in that medium only those organisms which can utilize both the nitrogen and carbon compounds present. For example, a medium which contains the necessary nutrient mineral salts and ammonia as a source of nitrogen. with a formate as a source of energy will allow the development of only those organisms which can assimilate ammonia nitrogen and utilize a formate as a source of energy, and for the carbon of its cell construction. If an organism is present which can assimilate ammonia nitrogen but cannot utilize a formate as a source of energy and cell construction it cannot develop. Conversely, if an organism is present which can utilize the formate but not the ammonia nitrogen there will be no growth. Therefore, starting with the simplest nitrogen and carbon compounds we can arrange a series of media having a common source of nitrogen and a changing source of energy, or *vice versa*, having a common source of energy but a changing source of nitrogen. We have, therefore, the possibility of developing as many media as the product of the number of nitrogen sources multiplied by the number of nitrogen free energy compounds. To these may be added a large number of media which may be made from

compounds containing both energy and nitrogen. For such organisms as are not able to develop upon a medium containing one of the simplest nitrogen salts, and a non-nitrogenous compound as a source of energy, we may develop a suitable medium by using a solution containing the essential mineral salts to different portions of which we add a different nitrogenous compound until we find a nitrogenous compound upon which it will develop. However, we must exercise caution here, particularly in the employment of complex compounds for mixed floras, because as a general rule the more complex the compound the greater the number of species which can utilize the compound or its degradation products. It is by simplicity of compounds that we may hope to control the growth of the great mass of saprophytes.

Possible media may be grouped according to their energy requirements as follows:

THE AMMONIA GROUP
Ammonia as a source of energy, no other form of nitrogen.

THE NITRITE GROUP
Nitrite as a source of energy, no other form of nitrogen.

THE SULPHUR GROUP
Sulphur as a source of energy, + ammonia.

THE IRON GROUP
Iron as a source of energy, + ammonia.

THE CYANIDE GROUP
Cyanide as a source of energy (no other form of nitrogen).
Cyanide as a source of energy, + ammonia nitrogen.

NITROGEN FIXING GROUP (Oligonitrophilic)
Compounds of carbon with hydrogen and oxygen as sources of energy, no nitrogen added.

THE ALIPHATIC ACID GROUP
The Aliphatic acids as a source of energy, + ammonia nitrogen.

Monobasic	*Monobasic hydroxy*	*Monobasic ketonic*
Formic	Glycolic	Pyruvic
Acetic	d-lactic	Acetoacetic
Propionic	l-lactic	Levulinic
Butyric	Hydracrylic	
Isobutyric		
Isovaleric		
Normal Valeric		

Di-basic	*Hydroxy di-basic*	*Unsaturated di-basic*
Oxalic	Malic	Fumaric
Malonic	Tartaric (dextro)	Maleic
Succinic	Tartaric (levo)	Mesaconic
Iso Succinic (Methyl malonic)	Meso-Tartaric	Citraconic

Tri-basic
Citric
Aconitic
Tricarballylic

THE AROMATIC ACID GROUP

Aromatic acids as a source of energy, + ammonia nitrogen.

Benzoic
Anisic
Tannic
Phthalic
Phenylacetic
Cinnamic

Salicylic
Gallic
Quininic
Amygdalic
Mandelic

THE ALDEHYDE GROUP

Aldehydes as a source of energy, + ammonia nitrogen.

Formaldehyde
Acetic aldehyde
Propyl aldehyde
Butyl aldehyde
Butyl aldehyde, iso

Benzoic aldehyde
Cuminol
Cinnamic aldehyde
Salicylaldehyde
Vanillin
Piperonal

THE KETONE GROUP

Ketones as a source of energy, + ammonia nitrogen.

Aceton
Butanon
Pentanon

THE ALCOHOL GROUP

Alcohols as a source of energy, + ammonia nitrogen.

Monohydric alcohols
Methyl alcohol
Ethyl alcohol
Propyl alcohol, normal
Propyl alcohol, iso
Butyl alcohol, iso
Butyl alcohol, primary normal
Butyl alcohol, secondary normal
Amyl alcohol, iso primary
Amyl alcohol, active

Polyhydric alcohols
  Ethylene glycol
  Glycerol
  Erythritol
  Arabitol
  Xylitol
  Mannitol
  Dulcitol
  Sorbitol
  Persitol
  Volemitol
  Adonitol

Aromatic alcohols
  Benzyl alcohol

THE CARBOHYDRATE GROUP

Carbohydrates as a source of energy + ammonia nitrogen.

Monosaccharids

Trioses	*Tetroses*	*Pentoses*
Glyceric aldehyde	d and l-Erythrose	d and l-Arabinose
Dioxyacetone	d and l-Threose	d and l-Xylose
		l-Ribose
		l-Lyxose

Methyl Pentoses
  Rhamnose
  Fucose (Rhodeose)
  Chinovose

Hexoses
Mannitol series

  d and l-Glucose
  d and l-Mannose
  Fructose
  Sorbose

Dulcitol series
  d and l-Galactose
  d and l-Talose
  Tagatose

Heptoses	*Octoses*	*Nonoses*
Mannoheptose	Mannoctose	Mannononose
Glucoheptose	Glucooctose	Glucononose
Galacto heptose	Galactoose	

Disaccharids	*Trisaccharids*	*Tetrasaccharid*	*Polysaccharids*
Maltose	Mannotriose	Stachyose	Dextrins
Iso maltose	Rhamninose		Soluble starches
Gentiobiose	Raffinose		Starches, Inulin,
Cellobiose	Gentianose		etc.
Lactose	Melicitose		Gums
Iso lactose			
Melibiose			
Turanose			
Sucrose			
Trehalose			

THE NON-NITROGENOUS GLUCOSIDE GROUP

Non-nitrogenous Glucosides as a source of energy, + ammonia nitrogen.

Phenolic glucosides	*Oxycumarin glucosides*
Arbutin	Aesculin
Methylarbutin	Daphnin
Phlorhizin	Fraxin
Glycyphyllin	*Oxyanthraqunone glucosides*
Hesperidin	Ruberythrinic acid
Naringin	Rubiadin glucoside
Iridin	Frangulin
Baptisin	*Oxyflarone glucosides*
Alcoholic glucosides	Apiin
Salicin	Fustin
Populin	Quercitrin
Coniferin	Sophorin
Syringin	Xanthorhamnin
Aldehydic glucosides	*Miscellaneous glucosides*
Helicin	Saponins
Salinigrin	Digitonin
Acidic glucosides	Digitalin
Convolvulin	Saponarin
Jalapin	Camatambin
Strophantin	
Gaultherin	

THE ESTER GROUP

Esters[2] as a source of energy, +ammonia nitrogen.

The entire series, after the first few types, may be repeated three times by substituting in place of the ammonia first, nitrite, -second, nitrate, -third, cyanide nitrogen. We would have then, nitrite-aliphatic-acid group; nitrite carbohydrate group; etc., or the nitrite series; and the nitrate aliphatic acid group and nitrate alcohol group, etc., or the nitrate series and cyanide-aliphatic-acid group and cyanide aldehyde group, etc., or the cyanide series.

Should occasion arise we may exclude all forms of nitrogen and carbon from the solution and substitute a series of amino-acids, amides, amino compounds, ureides, proteins, nitrogenous, glucosides, or cyanogen compounds, and thus build up series of media with each respective group.

[2] The utility of this group is doubtful, it being probable that if one of the constituents of the ester is available to an organism the ester may be also.

PREPARATION OF MEDIA

Conditions

All water used was double distilled from glass retorts and condensers. All chemicals used were the purest the market afforded. Each chemical was tested for impurities before use. All measures of growth were macroscopic.

Tests with liquid solutions were made in test tubes. Tests with solid media were made in Petri dishes. The incubator temperature adopted was 28°C. All media used were made neutral to phenolphthalein. The influence of sterilization by heat on composition and structure may be eliminated in most instances where an acid is used as a source of energy by preparing a medium in such a manner that it automatically sterilizes itself. This will be explained under preparation of media. Whenever this has not been possible the compound whose structure is liable to be influenced by high temperatures, has been sterilized by itself in neutral aqueous solution and added to the other sterile constituents, in correct proportion, by means of a sterile pipette. All transfers were made from cultures 48 to 96 hours old grown on standard agar.

Solid synthetic media

It will be necessary to describe the solid media first in order to explain the reason for the concentration of the liquid media. After numerous trials with starch, cellulose, aluminum hydroxide, iron hydroxide and washed agar as agglutinants, it was finally demonstrated that silica jelly was the most suitable solid medium. The silica jelly was made by a modified "Stevens Temple Method." (Centbl. Bakt., etc., II abt., vol. 21, 1908, p. 84.)

The method consisted essentially of dissolving c. p. K_2SiO_3 and c.p. Na_2SiO_3 in water in sufficient amounts to give a concentration of 34.2732 grams of H_2SiO_3 per liter. One half this concentration of H_2SiO_3 per liter gives a medium which will solidify in approximately five minutes, thus making a medium suitable for plating. The mixture of sodium and potassium sili-

cute gives us sodium and potassium salts in the final medium instead of only sodium salts, thereby lessening the danger of too great a concentration of sodium salt.

The detrimental influence of too great a concentration of the sodium and potassium salts can be still further lessened by using a mixture of acids; for example, we may use equivalent solutions of HCl, H_2SO_4 and H_3PO_4; thus giving in the finished medium chlorides, sulphates and phosphates of both sodium and potassium. Experiment demonstrated that the "Stevens Temple Method" might be still further modified by eliminating the $MgCO_3$ or Na_2CO_3.

The time of precipitation and gelatinization of H_2SiO_3 depends largely on two factors, first, reaction and second, concentration. Gelatinization is delayed or entirely prevented by either an excess of acid or an excess of the Na_2SiO_3 or K_2SiO_3. The most rapid gelatinization with any concentration takes place in a neutral solution.

The solid synthetic media were prepared in the following manner: Solutions of HCl, H_2SO_4 and H_3PO_4 were each standardized separately against the Na_2SiO_3 and K_2SiO_3 solution, so that 1 cc. of each acid would just neutralize 1 cc. of the silicate solution. Whenever an organic acid such as formic, acetic, lactic or tannic, etc., was used as a source of energy it was made in sufficient concentration to just neutralize an equal volume of the silicate solution. These acids, that is the HCl, H_2SO_4, H_3PO_4, and the organic acid (let us say acetic) were then mixed together in such proportion that the resulting salts from the sodium and potassium silicate would be present in the final silicate medium in quantities, approximately, inversely proportional to their osmotic action, thus giving a minimum osmotic pressure. Before standardizing the HCl there was added to it 0.5 gram $MgSO_4$, 0.01 gram $CaCO_3$ or CaO, 0.01 gram of $Fe_2(SO_4)_3$ and 0.01 gram of Mn SO_4, per liter. Ammonia nitrogen was added to the HCl as ammonium sulphate. Cyanide nitrogen was added to the HCl as potassium ferricyanide. Nitrite nitrogen was added to the neutral solution in the Petri dish, nitrate nitrogen was added to the acid mixture

as HNO_3. All the sources of nitrogen[3] were added in proportion to give one gram of their respective salts per liter. The mixture of acids was then placed in a sterile flask plugged with cotton and the flask connected with an automatic burette so that the burette would fill by siphon. The silicate solution was placed in another sterile cotton plugged flask and connected with another automatic burette, so that it too would fill by siphon. Each burette was allowed to fill and then stand several hours before use, so as to sterilize completely the flasks and burettes. The overflow cup of the burette was plugged with cotton, to prevent contamination from the air during titration. When sterile, 5 cc. of the acid mixture was added to a sterile Petri dish, after which there was added 5 cc. of the silicate solution. The plate was then rotated to mix the two solutions thoroughly, and then inoculated. If a non-acid compound was used as a source of energy (say glucose) a sufficient amount of a sterile aqueous solution, to give 10 grams per liter, was added at this point. Numerous tests proved both the acid and silicate solutions to be sterile in less than one hour. The resulting silica medium was neutral to phenolphthalein and set firmly in approximately five minutes. When a compound other than an acid was used as a source of energy it was added from a sterile aqueous solution to the medium in the Petri dish just before inoculation. The growth of organisms upon this solid medium resulted generally in quite typical colonies, although with a few organisms somewhat peculiar developments took place.

The liquid media used had a concentration similar to the above with the exception that H_2SiO_3 was absent. It is possible that some organisms which failed to grow might have grown in a lesser concentration. This was not determined because the first consideration was to develop solid synthetic media, and a more dilute silicate solution would not solidify soon enough; therefore the liquid media used as a check for the solid media must necessarily be of the same concentration. Parenthetically, it may be stated that the results of the liquid

[3] In future experiments, the availability of different salts of ammonia, nitrite, nitrate and cyanid must be tested..

and solid media did not always agree. The acetic ammonium medium will give an approximate idea of the composition and concentration.

Acetic-ammonia-silicate medium

H_2SiO_3	17.1366
NaCl	1.1620
Na_2SO_4	2.8209
$NaC_2H_3O_2$	1.6343
$Na_2H\ PO_4$	4.2330
K Cl	3.2954
K_2SO_4	7.7024
$K\ C_2H_3O_2$	4.3357
$K_2H\ PO_4$	11.5500
$MgSO_4$	0.5000
CaO	0.0100
$Fe_2(SO_4)_3$	0.0100
MnSO	0.0100
$(NH_4)_2SO_4$	1.0000
Water double dist	1000.0000

The acetic ammonia solution agrees with the above, excepting that it does not contain the H_2SiO_3, It may be made as follows: Make a solution of KOH and one of NaOH so that 1 cc. of each alkali will just neutralize 1 cc. of one of the acids which was standardized against the silicate solution. The KOH and NaOH solutions are then mixed in such proportions that when the mixture is brought in contact with the acid mixture the resulting sodium and potassium chlorides, sulphates, phosphates and acetates are present in quantities inversely proportional to their osmotic action. The acids may be prepared as previously described. The alkali mixture and the acid mixture may be placed in their respective bottles and connected by siphon with automatic burettes. Allow the burettes to fill as with the silicate medium and when sterile equal quantities of the alkali mixture and of the acid mixture may be run from the burettes in a suitable vessel.

It must be borne in mind that when glucose or an alcohol, or any other non-acid compound is used as a source of energy the proportion of each of the remaining salts increases. Likewise, the relative proportion changes when another acid, such as formic or lactic, is used in place of acetic acid. This fluctua-

tion of the chloride, sulphate and phosphate content of the medium might be eliminated in some instances[4] by adding the organic acids in such quantities that the resulting organic salt of sodium and of potassium should always contain equivalent quantities of these bases. Such a procedure might, however, give greater fluctuations in the osmotic action than the former procedure. This question, with many others, must be left for future investigators.

For convenience there is given below a concise method for making the ammonia-acetate-silicate medium and the ammonium acetate solution. These figures give quantities of salts which are only approximately inversely proportional to their osmotic action. Up to the present time the writer has been unable to secure all the necessary ionization constants.

Weigh out: 8.40 grams of c.p. Na_2SiO_3, 24.00 grams of c.p. K_2SiO_3 and dissolve in 500 cc. of distilled water. Dilute HCl to a concentration so that 1 cc. of the silicate solutions does not quite neutralize 1 cc. of the HCl. Add to the HCl 0.5 gram of $MgSO_4$, 0.01 gram of CaO, 0.01 gram of Fe_2SO_4 0.01 gram of $MgSO_4$ and 1 gram of $(NH_4)_2 SO_4$, and standardize the resulting HCl solutions against the silicate, using methyl orange as indicator, so that 1 cc. is equivalent to 1 cc. of the silicate solution.

Standardize a solution of H_2SO_4 in the same way, omitting the salts.

Standardize H_3PO_4 and CH_3COOH in a similar manner omitting the salts and using phenolphthalein as indicator.

The acids may then be mixed in the following proportion:

	cc.
HCl	153.5
CH_3COOH	153.5
H_2SO_4	77.0
H_3PO_4	116.0

One cubic centimeter of this acid mixture will just neutralize 1 cc. the silicate mixture, using phenolphthalein.

[4] Acids whose solubility in water is so low that solutions, equivalent to the silicate solution, cannot be obtained will still further complicate the question.

Ammonia-acetate solution

Prepare a N/0.2578 solution of NaOH and a N/0.6205 solution of KOH. Mix these solutions in equal proportions and substitute in the place of the silicate solution. The procedure from here on is the same as for the silicate medium.

Results with pure cultures

It has been the object in working with these media to test as many pure cultures of microorganisms upon each medium as could be obtained. In this way we shall be able eventually to determine all the compounds which each organism can use as a source of energy as well as those from which it can obtain its nitrogen. This will eventually enable us to group the known organisms (yeast and molds as well as bacteria) according to their ability to utilize energy material of a given chemical structure and configuration. The same thing applies to their nitrogen requirements. When this has been accomplished we may then eliminate all media suggested in the above groups that are common to a given group of organisms and retain for practical use the one which is the most serviceable. Thus eventually the above group of synthetic media will be reduced for general use to a few which are mutually inclusive and exclusive with occasional employment of others for special studies.

Several of the media described above have been tested upon 225 pure cultures of bacteria, 50 cultures of molds and 6 cultures of yeast. In the following table will be found the positive results obtained from three glucose media and three acetate media; only positive results are given because of lack of space.

Out of 225 pure cultures of bacteria tested the following results were obtained: On the glucose-ammonia medium there were 83 positive growths; on the glucose-nitrate medium 70 positive growths; on the acetate-ammonia medium 25 positive growths; and on the acetate-nitrate medium 17 positive growths. With the glucose-cyanide medium there were 7 positive growths; and with the acetate-cyanide medium there were 2 positive growths.

Positive growths on glucose ammonia, glucose nitrate, glucose cyanide and acetate ammonia, acetate nitrate and acetate cyanide media

NUMBER OF ORGANISM	GLUCOSE			ACETATE		
	NH$_3$	NO$_3$	CN	NH$_3$	NO$_3$	CN
7		X				
10						
15		X				
17		X				
18	X	X				
22	X	X		X		
23	X	X		X		
29	X	X		X	X	
30	X	X			X	
37	X					
40		X	X			
47	X	X		X	X	
52	X	X		X		
56						
59			X	X		X
72		X				
80		X				
88	X	X		X		
103	X	X		X		
110		X				
112	X	X		X		
116		X		X		
121		X				
122		X		X		
123		X		X		
124	X	X				
125	X	X		X		
126	X	X				
130	X					
135	X	X		X		
136	X	X				
137	X	X			X	
141	X					
142	X				X	
143		X	X			
144	X					
152	X	X		X	X	
161	X	X		X		
170	X	X		X		
175	X	X				
183		X		X		

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NUMBER OF ORGANISM	GLUCOSE			ACETATE		
	NH₃	NO₂	CN	NH₃	NO₃	CN
184	X		X	X		
185	X	X				
187	X	X		X		X
193		X				
195	X	X				
197		X				
224		X				
229	X					
230		X				
232	X	X				
235	X					
239	X	X				
240	X	X				
242	X	X				
244		X				
246	X					
257	X	X				
277	X					
282	X					
304	X	X				
306	X	X				
318		X				
242	X					
347		X				
352	X					
370	X	X				
371	X					
373	X					
374	X					
378		X				
380		X				
383	X					
385		X				
405		X				
424	X	X				
429	X	X			X	
436	X	X				
439		X				
468	X		X			
475		X				
477	X					
478	X					
487	X					
491		X				

NUMBER OF ORGANISM	GLUCOSE			ACETATE		
	NH₃	NO₃	CN	NH₃	NO₂	CN
492	X					
495	X					
497	X					
505	X					
506		X				
527	X	X				
528	X					
539	X					
540	X					
541	X					
544	X					
549	X	X				
555	X	X				
572	X	X				
573		X				
574	X					
575	X					
579	X					
583	X	X				
589	X	X				
592	X					
593	X	X				
594						
595	X					
596	X					
598	X					
601	X					
602					X	
603						
610						
612					X	
614					X	
615						
617		X				
618					X	

Laboratory numbers have been used purposely because the writer is not positive concerning the identity of some of the organisms used in the test. It must be borne in mind that the above results would be even more striking if the negative growths were included.

Space does not permit the presentation of more data, but the

above table demonstrates clearly the possibilities of the proposed synthetic media. It is apparent at once that the pure species of bacteria included in these experiments may be grown on some of the above synthetic media and controlled, to a certain extent, at least, by changing the energy or nitrogen source.

SUMMARY

The above scheme differs radically from the attempts to secure a universal medium, i.e., one upon which most bacteria of the saprophytic or parasitic groups will grow. It is on the contrary an attempt, by the use of definite sources of energy and definite sources of nitrogen, to exclude all species but those which can use the particular source of energy and nitrogen included in each case. If we are thus able to devise a series of mutually inclusive and exclusive media, we shall, after having tested the known species upon each, be able to state positively that any growth which appears upon any given medium is a member or members of a limited number of types belonging to that group (designated by media). Furthermore, we shall be able to plate out a soil, a milk, a water, etc., on a series of media and know, not only the number of bacteria present but also the number of the different groups present.

The schematic arrangement presented is tentative and is limited to water soluble compounds. Many of the groups suggested may have no energy value or the chemicals used may be too toxic or too expensive for practicable purposes. Other groups and compounds will no doubt be suggested by further study. Likewise, it is possible that subdivisions of the above groups may be made by using different sources of ammonia, nitrate or cyanide, etc., or by modifying other nutrient constituents or by changing the reaction. Up to the present time 16 of the above media have been tested. One using oxalate as a source of energy was negative throughout, the others have given promising results. It is hoped, therefore, that these tentative statements will stimulate needed investigation along this line, and criticism is heartily invited.

ON THE SIGNIFICANCE OF THE VOGES-PROSKAUER REACTION[1]

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Theobald Smith (1895) first called attention to the ratio of the gases evolved in the decomposition of glucose by *B. coli* and its relatives.   He pointed out that whereas *B. coli* produced twice as much hydrogen as carbon dioxide, equal volumes of these gases were formed by *B. aerogenes*.[2]   In consequence of the inaccuracies in the determination of the gases in the Smith tube, the gas ratio has been generally discarded as a differential criterion.   However, the comparatively recent work of Harden in England, and particularly that of Rogers and his associates in the Dairy Division of the United States Department of Agriculture, indicates that the gaseous and other decomposition products of glucose, if accurately determined, are of considerable importance in the differentiation of coli-like bacteria.

In a careful quantitative study of glucose fermentation, Harden and Walpole (1905), showed that *B. coli* evolved carbon dioxide and hydrogen in approximately equal volumes, and not, as had been observed by Smith, in the ratio of 1 to 2.   On the other hand, the *B. aerogenes* formed twice as much carbon dioxide as hydrogen instead of the equal volumes observed with the Smith tube.   They point out that the difference between the gas ratio obtained with the Smith tube and their accurately determined ratio is due to the loss of carbon dioxide in the former case owing to its solubility in the medium.

[1] Presented at the Seventeenth Annual Meeting of the Society of American Bacteriologists, Urbana, Illinois, December 28, 1915.

[2] *B. aerogenes* as employed in this paper, is synonymous with *B. lactis aerogenes*, i.e., those organisms of MacConkey's type IV (sucrose +, dulcitol −) which give a positive Voges-Proskauer reaction.

The real significance of the accurately determined gas ratio was not appreciated until 1914 when Rogers called attention to the striking correlation between this ratio and the source of the organisms. In three papers by Rogers, Clark and Davis, (1914) and Rogers, Clark and Evans (1914 and 1915), it is demonstrated quite conclusively that fecal strains of *B. coli* (at least those derived from bovine feces) break down glucose with the liberation of carbon dioxide and hydrogen in about equal volumes, while non-fecal (grain) strains form two or more times as much carbon dioxide as hydrogen. The sanitary significance of such a division is evident, but the accurately determined gas ratio is inapplicable to routine work.

Clark and Lubs (1915) note that the gas ratio is correlated with the H^+ ion concentration and that the difference in H^+ ion concentration between the low and high ratio groups is such that it may be easily recognized by methyl red. When grown in appropriate glucose media the low ratio, (fecal) group is acid and the high ratio (non-fecal) group alkaline to this indicator.

As no earlier investigators employed the methyl red reaction, the valuable work of these men cannot be compared directly or adequately with former investigations, unless some previously employed test is found which is well correlated with either the gas ratio or the methyl red test. The Voges-Proskauer reaction seems to serve this purpose.

THE VOGES-PROSKAUER REACTION

The chemistry of the Voges-Proskauer reaction has been worked out in detail by Harden and his associates in England. West refers to one of Harden's articles in which it is pointed out that the reaction is due to the production of acetyl-methyl-carbinol and urges that this test be studied further, as it is of considerable importance in recognizing *B. aerogenes* and *B. cloacae*. Among other investigators who have employed this reaction in studies on *B. coli* may be mentioned, Durham, Mac-Conkey, Rivas, Bergey and Deehan, Ferriera, Horta and Paredes, Copeland and Hoover, Clemesha, Archibald and more recently Kligler and Levine.

The significance of this reaction has not been fully appreciated by bacteriologists, nor has it been generally realized that the test is due to a definite end product of glucose fermentation. It will therefore not be amiss to review somewhat in detail the nature and chemistry of the Voges-Proskauer reaction.

The reaction takes its name from the fact that it was first observed by Voges and Proskauer in 1898, in their studies on the "Bacteria of Haemorrhagic Septicaemia." They describe the test as follows:

On addition of caustic potash, we observed a new and interesting color reaction. If the tube be allowed to stand 24 hours and longer at room temperature, after the addition of the potash, a beautiful fluorescent color somewhat similar to that of a dilute alcoholic solution of eosin forms in the culture fluid particularly at the open end of the tube exposed to the air. We have investigated a few of the properties of this coloring substance, which is not produced by the action of the alkali on the sugar, and have found that it is fairly resistant to the action of the external air. After a time however, it becomes paler, and finally gives place to a dirty greenish brown.

It has been repeatedly observed in this laboratory, that, with some cultures, a distinct coloration which may be observed about five hours after addition of the potash fades or disappears entirely after twenty-four to forty-eight hours.

In a study of the end products of the fermentation of glucose by *B. coli*, Harden and Walpole (1905–06) observed that the products ordinarily enumerated, (lactic, acetic, succinic and formic acids, ethyl alcohol and carbon dioxide) do not account for all of the carbon in the sugar. Aside from these substances, a crude glycol was also obtained. This crude glycol consists for the most part of 2:3 butyleneglycol (CH_3-CHOH-CHOH-CH_3). On oxidation it yields acetyl-methyl-carbinol (CH_3, CHOH.CO.CH_3), a volatile reducing substance, which, when mixed with potassium hydroxide in the presence of peptone, imparts an eosine-like coloration to the mixture on standing. Butyleneglycol is oxidized to acetyl-methyl-carbinol by *B. aerogenes*, but not by *B. coli*. Harden (1905) ascribes the Voges-Proskauer reaction to the production of this carbinol.

Walpole (1910) found that in the presence of oxygen *B. aerogenes* gave a larger yield of acetyl-methyl-carbinol from glucose and that fructose was decomposed in a similar manner.

Neither acetyl-methyl-carbinol nor butyleneglycol, when mixed with potassium hydroxide give the eosin-like coloration. In the presence of peptone, however, the coloration develops on standing in the case of the carbinol, but not with the glycol. According to Harden (1905) the reaction is due to the further oxidation of the carbinol ($CH_3CO.CHOH.CH_3$) to diacetyl ($CH_3CO.CO.CH_3$) which reacts with some constituent of the peptone. In a later study Harden and Norris (1911) report that in the presence of strong potassium hydroxide solution diacetyl reacts with proteins to give a pink coloration together with a green fluorescence. With arginine, creatine, dicyanamide and guanidine acetic acid, the pink coloration is also obtained but the fluorescence is absent. The reaction depends on the presence of the group $NH:C$ (NH_2) $N:HR$. The exact significance of R. has not been determined.

Among the organisms capable of forming acetyl-methyl-carbinol from carbohydrates may be mentioned, *B. aerogenes* Escherich, *B. cloacae* Jordan, *B. subtilis* Cohn, *B. vulgatus* Flügge. Peré obtained volatile substances which reduced Fehling's solution, by the aerobic fermentation of mannitol by *B. subtilis* and *B. vulgatus* and of glucose and glycerol by *Tyrothrix tenuis*.

CORRELATION OF VOGES-PROSKAUER AND METHYL-RED REACTIONS

A study of 167 coli-like bacteria obtained from various sources including raw and septic sewage, stock cultures from the American Museum of Natural History, and feces of the horse, cow, sheep, hog and man, showed that only those which were alkaline to methyl red (in a medium made up of 0.5 per cent $K_2 H PO_4$, peptone and glucose) gave the Voges-Proskauer reaction. Of 13 cultures which gave these reactions, 9 were from sewage and 4 from the museum collection. It should be noted that coli-like organisms giving these reactions were not obtained, even in a single instance, from the fecal samples.

In order to test further, the correlation between the H^+ ion concentration, and acetyl-methyl-carbinol production, 10 organisms which Dr. Kligler had observed to be positive for the Voges-Proskauer reaction were obtained. Two of these failed to form gas from glucose and will not be considered further here. The remaining eight strains were alkaline to methyl red and gave a positive Voges-Proskauer test.

Thirteen organisms were also obtained from L. A. Rogers. Five were acid to methyl red and gave a negative Voges-Proskauer test. One failed to grow at 37°C. All of the others (7) were alkaline to methyl red and gave a positive reaction when tested for the formation of acetyl-methyl-carbinol in glucose-peptone solution.

The high correlation between the Voges-Proskauer reaction and the indicator test of Clark and Lubs makes it possible to compare the work of Rogers and his associates with that of previous investigators. Such a comparison shows a striking unanimity of opinion as to the significance of these reactions. Rogers regards the high gas ratio and alkalinity to methyl red as characteristic of *B. aerogenes*-like bacteria. Practically all who have employed the Voges-Proskauer reaction have pointed out that this test is characteristic of *B. aerogenes* and B. *cloacae*. Of a large number of coli-like strains examined by MacConkey in 1905, only three *B. aerogenes* Escherich *B. copsulatus* Pfeiffer and *B. cloacae* Jordan gave the Voges-Proskauer reaction. Durham in 1901 observed that this test was given only by those organisms which he regarded as belonging to the *B. aerogenes* group.

THE DISTRIBUTION OF COLI-LIKE ORGANISMS WHICH GIVE A POSITIVE VOGES-PROSKAUER REACTION

Coli-like organisms which form acetyl-methyl-carbinol in glucose peptone solution are rarely found in feces. A reasonably accurate and reliable idea as to the distribution of such bacteria in nature may be obtained from a study of the distribution of *B. aerogenes* and *B. cloacae.*

MacConkey (1905) remarks on the scarcity of *B. aerogenes* in human feces. In the examination of 205 coli-like strains obtained from 22 samples of human stools, only 4 were *B. aerogenes* and of these 3 were isolated from a single sample. His observations on cow dung also indicated that this organism was extremely rare.

Ferriera, Horta and Paredes in an examination of 117 lactose fermenting strains from human feces obtained a positive Proskauer (presumably Voges-Proskauer) reaction in only eight instances. Among 81 coli strains obtained from 46 species of animals (8 birds and 38 mammals) they found only two which gave a positive "Proskauer" test.

The work of Clemesha is particularly significant because his conclusions are based on such large numbers of cultures. He examined 1207 organisms from human feces and 1029 from cow dung. In the latter *B. aerogenes* was found to be present in very small numbers and *B. cloacae* was sometimes common. In human stools, however, *B. aerogenes* and *B. cloacae* were very rare, nor was a sudden increase in the prevalence of these types ever observed. These findings are confirmed to a considerable extent by R. G. Archibald of the Wellcome Tropical Research Laboratories in an investigation of the water supply of Khartoum.

Of 117 cultures isolated in this laboratory from fecal sources (cow, horse, sheep, pig and man) not a single organism proved to be *B. aerogenes*, but of 39 organisms obtained from raw and septic sewage 9 (23 per cent) were of the *B. aerogenes* group (V.-P pos.). The relative abundance of these Voges-Proskauer positive organisms in sewage coupled with their extreme scarcity in human and other animal feces leads to the inference that they may perhaps represent soil forms.

Clemesha found that in India *B. aerogenes* is more prevalent in rivers and lakes during the wet reason than during the dry period. He explains this phenomenon on the basis of a supposed multiplication of the organisms in water, but observes that all his experiments indicate that such multiplication does not take place, at least in artificial mixtures. Nevertheless he maintains that in large bodies of water, such as rivers and lakes, there is un-

doubted multiplication of *B. aerogenes*. As to the prevalence of this organism he states, "In rivers, the period of time when rain is common is characterized by enormous increase in the number of *lactis-aerogenes* and yet we are perfectly certain that the organism is rare in feces."

These observations may be easily explained on the assumption that *B. aerogenes* is a soil form. In a study of coli-like microorganisms of the soil, now under way by B. R. Johnson and the author, preliminary tests have shown that a large proportion of cultures react positively for the Voges-Proskauer test, and are therefore of the *aerogenes-cloacae* group.

ON THE FORMATION OF ACETYL-METHYL-CARBINOL FROM DIFFERENT CARBOHYDRATES AND ALCOHOLS

Acetyl-methyl-carbinol, like carbon dioxid and various acids is a product of carbohydrate metabolism. The fermentation of carbohydrates with acid and gas formation is generally accepted as a reliable basis for differentiation of *B. coli*. It is possible that a study of the production of acetyl-methyl-carbinol from various carbohydrates might also be of differential significance.

Harden and Norris obtained acetyl-methyl-carbinol by growing *B. aerogenes* or *B. cloacae* in peptone solutions containing glucose, fructose, mannose, galactose, arabinose, isodulcite, mannitol or adonitol, but this compound was not formed with glycerol ethyleneglycol, or acetaldehyde.

Ferriera, Horta and Paredes, studied the Proskauer reaction (presumably the Voges-Proskauer reaction) with glucose, galactose, maltose, lactose, sucrose, dulcitol, mannitol, and inulin. The reaction was positive eight times out of 117 tests with glucose, 7 times out of 48 tests with galactose, and twice in 48 tests with mannite. Practically all cultures gave traces with lactose and maltose while with dulcitol and inulin the reaction was invariably negative. These authors give an interesting table in which *B. coli*, *B. cloacae* and *B. aerogenes* are differentiated on the basis of the "Proskauer" reaction with different carbo-

hydrates and alcohols. It appears that *B. cloacae* differs from *B. aerogenes* by the ability to give the reaction with mannite and galactose while the latter (*B. aerogenes*) may be distinguished from *B. coli* by its positive reaction in glucose and saccharose. The differentiation is suggestive and interesting but questionable, since the experimental evidence is inconclusive.

The term "Voges-Proskauer Reaction" is generally understood to mean the production of an eosin-like coloration when a glucose broth culture is made alkaline with potassium hydroxide. To employ the same term when some other carbohydrate is substituted for glucose may lead to confusion. It is therefore, suggested that the term "Voges-Proskauer Reaction" be restricted to designate the formation of acetyl-methyl-carbinol from glucose, but when referring to its production from other carbohydrates or alcohols, the term *Acetyl-methyl-carbinol Test* or merely *Carbinol Test* be employed. The nature of the substance being tested for is thus indicated just as is the case with the *Indol Test*.

The following experiment was carried out as a preliminary study on the production of acetyl-methyl-carbinol from various substances by coli-like bacteria. Forty-six organisms were selected. Twenty were strains which previous studies had shown did not produce the carbinol from glucose. They represent five sources, horse, sheep, cow, pig and man. From each source a culture representative of each of MacConkey's four groups was included. As far as possible no two cultures were from the same animal. The object of this selection was to obtain a group of Voges-Proskauer negative strains which would be likely to contain many different varieties.

The other 26 cultures were strains which previous tests had shown could form the carbinol from glucose. These included 9 strains isolated from sewage, and 17 strains obtained from Rogers and Kligler.

The organisms were inoculated into a medium consisting of 0.5 per cent di-potassium phosphate 0.5 per cent peptone and 0.5 per cent of the test substance. The Digestive Ferments Company peptone was employed for the test with glucose.

With all other test substances Wittes peptone was used. Incubation was at 37°C. for seventy-two hours.

Of the 26 supposedly Voges-Proskauer positive organisms, 4 failed to give the reaction in this experiment. Whether this phenomenon is due to the difference in peptone, variation in period of incubation, or loss of physiological function needs to be further investigated. With one of these organisms, it was observed, about eight months ago, that the test for acetyl-methyl-carbinol was negative until the seventh day of incubation.

In the table below are summarized the results. The cultures are arranged in three groups. One group comprises those strains which, repeated tests have shown, do not form acetyl-methyl-carbinol from glucose, even on long incubation (7 days). Another group contains 22 strains which do form this carbinol from glucose. The third group includes the 4 cultures whose Voges-Proskauer reaction is questionable.

Correlation between the formation of acetyl-methyl-carbinol in glucose peptone solution and in peptone solution containing other carbohydrates and alcohols

TEST FOR ACETYL-METHYL-CARBINOL IN GLUCOSE PEPTONE SOLUTION (VOGES-PROSKAUER REACTION)	POSITIVE (22 STRAINS)		NEGATIVE (20 STRAINS)		QUESTIONABLE (4 STRAINS)	
	No.	Per cent	No.	Per cent	No.	Per cent
Positive reactions with:						
Fructose	22	100.0	0	00.0	0	00.0
Galactose	20	90.9	0	00.0	1	25.0
Maltose	21	95.5	17	85.0	2	50.0
Lactose	15	68.2	5	25.0	0	00.0
Sucrose	21	95.5	1	5.0	1	25.0
Raffinose	8	36.4	0	00.0	1	25.0
Mannitol	13	59.1	0	00.0	0	00.0
Glycerol	0	00.0	0	00.0	0	00.0
Salicin	5	22.0	0	00.0	0	00.0
Dextrin	0	00.0	0	00.0	0	00.0

Practically all strains gave a trace of acetyl-methyl-carbinol with maltose. A very striking fact indicated in the table is that coli-like organisms which do not form acetyl-methyl-carbinol from glucose are characterized by an inability to produce this compound from the other substances tested. In only

one instance (5 per cent) was the carbinol test positive with sucrose; in 5 cultures (25 per cent) traces were observed with lactose; but with fructose, galactose, raffinose, mannitol and salicin the reaction was invariably negative.

On the other hand, the carbinol test with the organisms which gave a positive reaction with glucose was almost always positive with levulose (100 per cent), galactose (90.9 per cent), and sucrose (95.5 per cent), usually positive with lactose (68.2 per cent) and mannitol (59.1 per cent), and occasionally positive with raffinose (36.4 per cent) and salicin (22 per cent).

These results show quite conclusively that the metabolism of certain carbohydrates by the fecal group of coli-like organism is fundamentally different from that of the non-fecal group.

The carbinol test was usually most intense and distinct in sucrose peptone solution.

CONCLUSIONS

The Voges-Proskauer reaction and alkalinity to methyl red in 0.5 per cent di-potassium phosphate, glucose peptone solution are correlated. These reactions were not given by any of the 117 strains isolated from the feces of the horse, cow, sheep hog and man.

A review of the literature on the distribution of *B. aerogenes* and *B. cloacae* (Voges-Proskauer positive organisms) corroborates the contention of Rogers and his associates that coli-like organisms which give a high CO_2/H_2 gas ratio, and an alkaline reaction to methyl red in 0.5 per cent peptone di-potassium phosphate glucose solution, are rare in feces.

The natural habitat of coli-like bacteria which form acetylmethyl-carbinol from glucose and other carbohydrates is probably the soil.

The production of acetyl-methyl-carbinol from different carbohydrates and alcohols might serve as a differential index.

Practically all strains gave a trace of the carbinol in maltosepeptone solution.

Of the organisms which did not give the Voges-Proskauer reaction only 5 per cent formed acetyl-methyl-carbinol from sucrose, and 25 per cent gave a trace from lactose. With all

the other substances tested fructose, galactose, raffinose, mannitol, glycerol, salicin, and dextrin the carbinol test was negative.

Of the cultures which were positive for the Voges-Proskauer reaction, 100 per cent formed the carbinol from fructose, 90.9 per cent from galactose, 68.2 per cent from lactose, 95.5 per cent from sucrose, 36.4 per cent from raffinose, 59.1 per cent from mannitol and 22 per cent from salicin.

The Voges-Proskauer reaction is of considerable sanitary significance. It differentiates between fecal and non-fecal coli-like organisms and may be an index of soil washings.

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STUDIES ON SOIL PROTOZOA AND THEIR RELATION TO THE BACTERIAL FLORA. II[1]

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VI. THE EFFECT OF VOLATILE ANTISEPTICS UPON SOIL PROTOZOA

Introduction

It is claimed by Russell and Hutchinson and their co-workers that soils partially sterilized with volatile antiseptics are entirely freed from protozoa. Hutchinson (1913) further claims that the larger types of the soil protozoa are killed by the treatment of soil with caustic lime. On the other hand, the results reported by Gainey (1912) and by Grieg-Smith (1911) indicate that the application of such amounts of volatile antiseptics as are used in practice does not exterminate the protozoa. Even if it be acknowledged that some types of the soil protozoa are able to resist the process of partial sterilization by antiseptics, we must still consider the contention of Russell and Hutchinson that the harmful factor is inactivated for a considerable period, when not exterminated. Further, the possibility exists that the kinds of protozoa most detrimental to the bacterial flora are peculiarly susceptible to the antiseptics. Since the greater part of the protozoan fauna of the soil is inactive, the mere survival of certain types is not necessarily important, but the effect of volatile antiseptics upon the *active soil protozoa*, on the other hand, would appear significant.

Experiments

Tests were made of partially sterilized soils to determine the number of protozoa and also the types. These tests were made

[1]Continued from the Journal of Bacteriology, vol. i, no. 1, p. 35.

with pots each containing one kilogram of soil. The number of protozoa was determined by the dilution method, while the types were determined by the inoculation of 25 grams of soil into sterile hay infusion.

The effect of volatile antiseptics upon the active protozoa was determined by the treatment of soils with carbon bisulphide and toluene and by determining the number of protozoa one day after treatment and again after two months. The results (Table XXVI) show that the active protozoa are not exterminated and again multiply to numbers equivalent to those found in normal soils. *Monas sp.*, *Dimorpha radiata* and *Flagellate A* were all observed on the 1/10,000 dilutions after two months.

TABLE XXVI

Effect of toluene and CS$_2$ on the soil protozoa

POT NO.	TREATMENT	NUMBER OF PROTOZOA PER GRAM	
		1 day	60 days
1	2 per cent toluene...............	Less than 10	10,000
2	2 per cent toluene...............	Less than 10	10,000
3	2 per cent CS$_2$..................	Less than 10	10,000
4	2 per cent CS$_2$..................	Less than 10	10,000

In another experiment toluene, carbon bisulphide and chloroform were used, and samples were taken at the end of one month to determine the number and types of protozoa present. In this test it was found that the protozoan fauna had not been simplified, as far as could be noted by microscopic examination, there being present a very complex mixture of ciliates, flagellates and amoebae. At the end of a month the active protozoa were again present in just as large numbers as are found in untreated soils.

In the foregoing tests the antiseptics used were left in the soil. It was also thought desirable to treat some soils by the method followed by Russell and Hutchinson. These workers usually employed 1 per cent toluene and then after one day spread the soil out to allow the antiseptic to evaporate. Four pots of soil were treated after this manner and four other pots

TABLE XXVII

(Effect of toluene, CS_2 and $CHCl_3$ upon the soil protozoa (one month after treatment)

POT NO.	TREATMENT	PROTOZOA PER GRAM	TYPES OF PROTOZOA
1	2 per cent toluene..............	10,000	C. F. A.
2	2 per cent toluene..............	10,000	C. F. A.
3	2 per cent toluene..............	10,000	C. F. A.
4	2 per cent CS_2.	10,000	C. F. A.
5	2 per cent CS_2................	10,000	C. F. A.
6	2 per cent CS_2................	10,000	C. F. A.
7	2 per cent $CHCl_3$..............	10,000	C. F. A.
8	2 per cent $CHCl_3$..............	10,000	C. F. A.
9	2 per cent $CHCl_3$..............	10,000	C. F. A.

C = Ciliates; F = Flagellates; A = Amoebae.

were treated with 1 per cent toluene but not evaporated. As is shown in Table XXVIII the results after one month were similar to those obtained in the other experiments.

TABLE XXVIII

Effect of toluene left in and evaporated upon the soil protozoa (one month after treatment)

POT NO.	TREATMENT	PROTOZOA PER GRAM	TYPES OF PROTOZOA
1	Left in..........................	10,000	C. F. A.
2	Left in..........................	10,000	C. F. A.
3	Left in..........................	10,000	C. F. A.
4	Left in..........................	10,000	C. F. A.
5	Evaporated.....................	10,000	C. F. A.
6	Evaporated.....................	1,000	C. F. A.
7	Evaporated.....................	10,000	C. F. A.
8	Evaporated.....................	10,000	C. F. A.

Another test was made in which large amounts of volatile antiseptics were used in order to see if the protozoa could be entirely eliminated from soil. Soils were treated with 5 and 10 per cent of toluene and carbon bisulphide and the antiseptics left in the soil. Even with such large amounts of antiseptics the protozoa were not entirely eliminated, although the fauna was considerably simplified, especially with carbon bisulphide. As is shown in Table XXIX, ciliates, flagellates and amoebae

were found in every case except in the one treated with 10 per
cent carbon bisulphide in which no amoebae were observed.
In all of these soils *Monas sp.*, *Dimorpha radiata* and *Flagellates
A* and *B* were present.

TABLE XXIX

Effect of large amounts of toluene and carbon bisulphide upon the soil protozoa

TREATMENT	5 PER CENT TOLUENE			10 PER CENT TOLUENE			5 PER CENT CS₂			10 PER CENT CS₂	
Types of protozoa..........	C.	F.	A.	C.	F.	A.	C.	F.	A.	C.	F.

Several attempts were made without success to demonstrate
a stimulation of the protozoa, similar to that of the bacteria,
subsequent to the application of volatile antiseptics to the soil.
Moore (1912) in an address on the "Micro-organisms of the Soil"
stated that results obtained in his laboratory indicated that the
protozoa in soil not only withstood the action of antiseptics
but that they might be increased by such treatment. Wood-
ruff (1908) has shown that the multiplication of infusoria may
be stimulated by small doses of alcohol. The dilution method
for the determination of the number of protozoa is far too crude
to measure small differences so the fact that it failed to demon-
strate any increase in the number of protozoa following the
application of volatile antiseptics to soil cannot be considered
of much importance.

Discussion

From the results herein reported it may be concluded that
volatile antiseptics in the amounts used in practice do not free
the soil from protozoa. The active soil protozoa not only sur-
vive, but multiply rapidly and again attain their normal num-
bers, usually within a month after treatment. It is difficult
to explain the failure of Russell and Hutchinson to find protozoa
in the soils which they treated. They noted the survival of
certain flagellates which they do not, however, associate with
the "detrimental factor." The failure of these workers to find
ciliates and amoebae may be due to insufficient samples. The
ciliates and amoebae are greatly reduced by the treatment of

soil with volatile antiseptics; these organisms, being inactive in most soils, do not increase subsequently and so it is obviously necessary to use a larger sample in order to demonstrate their presence.

That the presence of protozoa in the partially sterilized soils used in this work was not due to contamination was shown by holding ten pots of sterilized soil under identical conditions for one month and then taking samples for protozoa. Nine of these pots were found to be free from protozoa, while the tenth contained one small flagellate.

These results argue strongly against the protozoan theory as an explanation of the phenomena of partial sterilization, but it cannot be said they positively disprove it, since, as was pointed out before, the particular kinds that are detrimental, if such exist, may be very sensitive to volatile antiseptics.

VII. EXPERIMENTS RELATING TO THE POSSIBLE EXISTENCE IN SOIL OF A HARMFUL BIOLOGICAL FACTOR WHICH IS DESTROYED BY THE ACTION OF VOLATILE ANTISEPTICS

Introduction

The experiments made in his part of the work were planned in an effort to determine whether the beneficial action of volatile antiseptics upon the soil bacteria is due to the destruction of a detrimental factor which is antagonistic to them. This problem was attacked in much the same way as was the study of the soils containing protozoa and free of protozoa (Part IV). If normal soils contain a bacterial-limiting factor while partially sterilized soils do not, it would seem that that fact could be quite definitely established by a comparison of the numbers of bacteria found in these soils when subjected to various conditions. It should also be easy to demonstrate the presence of this harmful factor by the reinfection of the partially sterilized soils with a small amount of untreated soil.

The effect of volatile antiseptics upon the subsequent development
of bacteria and protozoa in soil

It was thought that some light might be thrown upon the
protozoan theory by making bacterial and protozoal counts
on soils subsequent to treatment with volatile antiseptics. If
this theory is correct we would expect to find the greatest num-
ber of bacteria in partially sterilized soil at a time when the

TABLE XXX

Effect of volatile antiseptics upon the bacteria and protozoa in soil
Fifteen days after treatment

POT	TREATMENT	BACTERIA PER GRAM	PROTOZOA PER GRAM
1	Control.........................	15,000,000	20,000
2	Control.........................	14,500,000	20,000
3	2 per cent toluene..............	14,000,000	100
4	2 per cent toluene..............	15,000,000	1,000
5	2 per cent CS_2.................	13,000,000	100
	Thirty days after treatment		
1	Control.........................	20,800,000	20,000
2	Control.........................	20,200,000	20,000
3	2 per cent toluene..............	48,000,000	20,000
4	2 per cent toluene..............	49,300,000	20,000
5	2 per cent CS_2.................	44,400,000	20,000
	Forty-five days after treatment		
1	Control.........................	17,000,000	20,000
2	Control.........................	21,000,000	20,000
3	2 per cent toluene..............	45,000,000	20,000
4	2 per cent toluene..............	46,000,000	20,000
5	2 per cent CS_2.................	110,000,000	20,000

protozoa are depressed. This, however, does not appear to be
the case as is shown by the following experiment. Determina-
tions were made of the numbers of bacteria and protozoa in
treated and untreated soils at intervals of fifteen days after
treatment. The results of this test are given in Table XXX.

This table shows that the maximum number of bacteria is
not found while the protozoa are depressed, but rather that

the development of the two classes of micro-organisms subsequent to treatment with volatile antiseptics runs parallel. This experiment was verified by another test in which normal and carbon bisulphide-treated soils were compared. In this test (Table XXXI) the number of bacteria in the treated soil rose above that of the control soil by the fifteenth day, but at this period the protozoa in the treated soil had also returned to their normal level. It is seen also that the number of bacteria continued to increase after the protozoa had again become as numerous as in untreated soil.

TABLE XXXI

Effect of CS_2 upon the bacteria and protozoa in soil

POT	TREATMENT	FIFTEEN DAYS		THIRTY DAYS	
		Bacteria	Protozoa	Bacteria	Protozoa
1	Control...........	23,000,000	10,000	60,000,000	20,000
2	2 per cent CS_2.....	94,000,000	20,000	240,000,000	20,000

The reinoculation of partially sterilized soils

In their work at the Rothamsted Station Russell and Hutchinson (1913) claim to have demonstrated that the soil contains a detrimental factor since the bacterial content of partially sterilized soil may be reduced by reinoculation with untreated soil. It is pointed out that when soil treated with a volatile antiseptic is reinoculated with 5 per cent of untreated soil the number of bacteria is reduced, while if only 0.5 per cent of normal soil is added no such reduction takes place. These observations are explained by the assumption that when only 0.5 per cent of untreated soil is added the harmful factor is not transmitted, but when 5 per cent is used for the inoculum the treated soil again becomes infected with the undesirable group of organisms. The soundness of this view may certainly be questioned, as it is difficult to understand why it should be necessary to use such a large amount of untreated soil in order to insure the presence of a factor which is supposed to exist in amount sufficient to suppress the bacteria. A review of the work of Russell

and Hutchinson reveals the fact that in some of the tests the treated soils which were reinoculated with 5 per cent of untreated soil did not show an appreciable depression in the number of bacteria, and they qualify their conclusion on this point with the statement that, "the harmful factor is not invariably transmitted to the same extent from the untreated to the partially sterilized soil and in a few cases indeed it is not transmitted at all."

In the experiments which were carried out in this laboratory the partially sterilized soils were reinoculated with 1 per cent of untreated soil; since at least 1 kgm. of soil was used in each pot the inoculum never consisted of less than 10 grams of normal soil. It could hardly be doubted that this amount of soil would be sufficient to transplant the group of organisms, if such exist, which act as a limiting factor upon the bacterial flora.

The work which has been done on the reinoculation of partially sterilized soils (Tables XXXII to XXXIV) fails to give any indication that a harmful factor is thus introduced. It would appear, on the other hand, that if reinfection of the treated

TABLE XXXII

Effect of reinoculation of treated soil with untreated soil (treatment of 2 per cent toluene)

POT	TREATMENT	NUMBER OF BACTERIA PER GRAM		
		30 days	60 days	90 days
1	Control................	56,000,000	80,000,000	69,000,000
2	Control................	66,000,000	75,000,000	62,000,000
3	Reinoculated...........	57,000,000	82,000,000	79,000,000
4	Reinoculated...........	62,000,000	100,000,000	92,000,000

TABLE XXXIII

Effect of reinoculation of treated soil with untreated soil (treatment 1 per cent toluene: evaporated)

POT	TREATMENT	NUMBER OF BACTERIA PER GRAM	
		15 days	30 days
1	Control................................	149,000,000	95,000,000
2	Control................................	127,000,000	81,000,000
3	Reinoculated..........................	152,000,000	130,000,000
4	Reinoculated..........................	178,000,000	92,000,000

TABLE XXXIV

Effect of reinoculation of treated soil with untreated soil (treatment 2 per cent CS₂)

POT	CONTROL	AVERAGE	REINOCULATED	AVERAGE
1	273,000,000 ⎫		247,000,000 ⎫	
2	218,000,000 ⎬	255,300,000	317,000,000 ⎬	392,000,000
3	285,000,000 ⎭		422,000,000 ⎭	

Incubation period after reïnoculation: 2 months.

soil has any effect it is to increase the number of bacteria rather than to decrease it. However, the data on this point are doubtless within the boundaries of experimental error. It is difficult to reconcile these findings with the theory of Russell and Hutchinson.

The number of bacteria in partially sterilized and normal soils at different temperatures

One of the strongest points in the evidence produced by Russell and Hutchinson to prove that the soil contains a harmful biological factor was the difference in the behavior of untreated and partially sterilized soils when incubated at different temperatures. Their results indicated that the maximum development of bacteria in the untreated soil was at low temperatures (5° to 12° C.), while in treated soil the greatest number was found at 20°C., and at 30°C. there was a marked increase over that found at 12°C.—the maximum in the case of the untreated soil. This phenomenon they claim shows that the bacteria under normal conditions are limited by the detrimental factor and that their maximum development takes place under conditions unfavorable for the harmful factor.

This point has been tested by the comparison of toluened and normal soils at 10°, 22°, and 37°C. The treated soil used had been treated with 2 per cent toluene three months previously. These soils were incubated for one month at their respective temperatures and then sampled and their bacterial counts determined. The results are given in Table XXXV.

The number of bacteria in treated and untreated soils at different temperatures

TREATMENT	NUMBER OF BACTERIA PER GRAM		
	10° C.	22° C.	37° C.
Untreated......................	21,000,000	23,000,000	22,000,000
2 per cent toluene..............	64,000,000	49,000,000	36,000,000

These data are not sufficient to base any conclusions upon but it can not be said they indicate very much, either in favor of the protozoan theory or against it. It will be seen that the greatest difference in the numbers of bacteria in the treated and untreated soils was at 10°C., a point not in favor of the protozoan theory. On the other hand, the least difference was found at 37°C., which point may support the theory of Russell and Hutchinson.

It was decided to carry out another experiment at 37°C. in order to throw more light on this point. Instead of using soils which had been previously treated, the soils were first placed at 37°C. and allowed to incubate at that temperature for one month. Half of them were then treated with 2 per cent carbon bisulphide. If the protozoan theory is correct the antiseptic should have very little effect at this high temperature. One month after treatment bacterial counts were made. The results obtained are given in Table XXXVI.

TABLE XXXVI

Effect of CS_2 upon the number of bacteria in soil at 37°C.

POT	NUMBER OF BACTERIA PER GRAM			
	Untreated	Average	2 per cent CS_2	Average
1	21,000,000	21,000,000	208,000,000	228,000,000
2	21,000,000		248,000,000	

The results are very striking; a difference of over ten fold in the number of bacteria in the treated and untreated soils being found. This observation indicates strongly that the beneficial action of volatile antiseptics in soil is not to be explained by

its effect upon the protozoa. Soil extract and hay extract cultures made from untreated soil and incubated at 37°C. have failed entirely to reveal the presence of any of the active types of protozoa which have been mentioned as especially abundant in soil. In such cultures only a very few -types of protozoa appear at all and these only slowly and in small numbers.

The number of bacteria developing in sterilized soils reinoculated with untreated and with partially sterilized soils

The preceding experiments appear to demonstrate quite conclusively that the beneficial effect of volatile antiseptics in soil is not due to the destruction of a biological factor, unless it be assumed that the treatment of soil so changes it that the harmful organisms are no longer able to develop in it, even though it is reinoculated with them. An experiment was planned in order to see if this explanation is a true one. Two pots of sterile soil were inoculated with 1 per cent of normal soil, while two other pots were inoculated with 1 per cent of a soil which had been treated with 2 per cent toluene. In case the antiseptic really destroys a harmful factor that fact should be indicated by a much greater number of bacteria in the soils inoculated with the treated soil. This result, however, was not obtained; on the contrary, the counts made at thirty and forty-five days after inoculation showed no practical difference between the numbers of bacteria in the two soils, as is shown in Table XXXVII.

TABLE XXXVII

The number of bacteria developing in sterilized soils inoculated with normal and with toluened soils

POT	NUMBER OF BACTERIA PER GRAM			
	35 days		45 days	
	Normal	Toluened	Normal	Toluened
1	127,000,000	112,000,000	126,000,000	102,000,000
2	208,000,000	148,000,000	110,000,000	104,000,000
Average.......	190,000,000	130,000,000	118,000,000	103,000,000

*The effect of carbon bisulphide on the number of bacteria in sterilized
soils reinoculated with normal soil and with protozoa-free soil*

Another experiment performed in order to detect the presence
of the "harmful factor" was to inoculate soils sterilized by
steam with normal soil and with the protozoa-free soil described
in an earlier part of this paper.   These soils were allowed to
stand three weeks and were then treated with 1 per cent of car-
bon bisulphide.   Bacterial counts were made before the soils
were treated and then at fifteen and thirty days after treatment.
According to the phagocytic theory, it would be expected that
the number of bacteria in the soil inoculated with normal soil
would subsequently be greatly increased while the soils inoculated
with the protozoa-free soil should not be appreciably affected.

As in the previous experiments, the results of this test give
no indication that there exists in soil a biological factor which
is harmful to the bacterial flora.   It will be seen upon examina-
tion of Table XXXVIII that the soils free of protozoa and those
containing protozoa behaved in exactly the same way.

TABLE XXXVIII

*Effect of CS₂ upon sterilized soils inoculated with normal soil and with protozoa-
free soil*

| POT | NUMBER OF BACTERIA IN MILLIONS PER GRAM | | | | | |
| | Before treatment | | 15 days | | 30 days | |
	Without protozoa	With protozoa	Without protozoa	With protozoa	Without protozoa	With protozoa
1	178	120	228	140	166	109
2	172	110	182	142	144	91
Average......	175	115	205	141	155	100

The results of this test add further weight to the preceding
experiments all of which point to the non-existence of a detri-
mental biological factor in soil.   The fact that volatile antiseptics
have no appreciable effect in soils which have been sterilized
by steam and then reinoculated with normal soil would appear
to indicate that the beneficial effects derived by the use of these

substances are due to some action of the antispetics on the soil itself rather than to a simplification of its micro-organic population.

VIII. RÉSUMÉ

Discussion

The results of the foregoing experiments appear to establish quite definitely that protozoa in the soils which have been studied do not have a detrimental effect upon the bacterial flora. It is difficult to see how the action of an important phagocytic agent could have escaped detection by the methods employed unless the factor is unable to increase in soils which have been previously sterilized with steam or partially sterilized with volatile antiseptics when again introduced into these soils with an inoculum of normal soil. This restricted power of growth would be very different from the properties of microorganisms in general, either of animal or plant nature, and there is no evidence, as far as we are aware, that a group of organisms with such peculiar characteristics exists in the soil. As has been pointed out, the soil protozoa, at least those types which appear in liquid cultures, grow better in soil which has been previously subjected to steam sterilization just as do the bacteria. Aside from the evidence that soil does not contain a biological factor which is inimical to the bacterial flora, the facts that volatile antiseptics do not exterminate the soil protozoa, and that partial sterilization of soil under conditions unfavorable for the action of protozoa (e.g., at 37°C.) is followed by the characteristic rise in the number of bacteria, would appear to cast serious doubt upon the theory of Russell and Hutchinson as an explanation for the effect of volatile antiseptics upon the soil bacteria.

Cunningham (1914) has recently published the results of some work which he thinks proves that protozoa act as a limiting factor upon the bacterial flora in soil. The fact that his data on this point are derived from only two experiments, one of which gave negative results, would preclude his conclusions

from very serious consideration. A study of the methods he used indicates, however, that the difference found in the soils with and without protozoa might have been due to a difference in the complexity of the two flora, as was the case in the experiments reported in Part IV (see Table XI) of this paper. In fact, his manner of attack was very similar; sterilized soils were employed as a substratum, and inoculations were then made into these soils of cultures containing protozoa and free of protozoa. "One flask was inoculated with bacteria plus protozoa from a culture of protozoa from soil, the other received as nearly as possible an equal inoculation from the same culture of bacteria alone." It is not clear from this statement how he obtained the bacterial culture free from protozoa, but it is very certain that a protozoa-free culture could not be obtained which would contain as complex a bacterial flora as did the original culture from which it was derived. As was previously pointed out, a difference in the complexity of the bacterial flora in different soils may cause a great disparity in the counts obtained by the plate culture method. This fact was apparently not recognized by Cunningham as he concluded that "the reduction in bacterial numbers in the soils inoculated with protozoa is very marked and lies well outside the limits of experimental error." A review of the data in Part IV of this paper will show, on the contrary, that his results may fall well within the limits of experimental error.

It is believed that the conclusions drawn from the work herein reported will hold in general for the cultivated soils in this country, but it is not desired to make too broad an application of them. Many of the "sick" soils which have been studied at the Rothamsted Experimental Station are very different from the ordinary American soil. Martin and Lewin (1914) describe a sick cucumber bed which was made up of one part of light pasture soil, one part of heavy pasture soil and two parts of horse manure, and had an optimum moisture content of 62 per cent. The assumption that the biological conditions in such a soil are the same as in the average soils of the United States (which contain about 2 per cent organic matter and the optimum

moisture content of which is from 16 to 18 per cent) would be obviously unwarranted. That a difference in the micro-fauna does exist under various soil conditions is indicated by the fact that Martin and Lewin have found amoebae to be the predominating types of protozoa in the soils they have studied, which are very rich in organic matter, while the results reported here, as well as the data obtained by Cunningham on German soils, indicate that the flagellates occur in greater numbers than do the amoebae. It appears possible that in the rich soils and green-house beds, which have been studied extensively at the Rothamsted Station in connection with soil sickness, there might be a phagocytic agent which is not active in ordinary soils. This possibility, however, should not make us unmindful of the fact that no direct evidence has as yet been produced which indicates that such a factor exists in any cultivated soil. It should also be remembered that the beneficial effects of partial sterilization of soil—for the explanation of which the protozoan theory was advanced—have been observed in all localities in which the problem has been studied and in nearly all types of soil.

The question of the activities of the protozoa which lead an active existence in soil is a problem upon which much work could profitably be done. The active protozoa which occur in soils in large numbers certainly have functions there, some of which in fact may be very important. It is not desired to give the impression that because the protozoa which have been studied do not exert a limiting action on the bacteria in soil that it is thought that they do not ingest bacteria at all. Some in all probability do not, while others (e.g., *Monas*) it would appear undoubtedly do. Why active protozoa which feed upon bacteria should not cause a measurable decrease in the number of bacteria in soil is difficult to explain. It would seem that the excretory products of the protozoa which feed upon the soil bacteria would increase the amount of available energy for the rest of the bacteria so that a condition of metabiosis would be established which might offset the antagonistic action of the protozoa. This hypothesis does not appear unreasonable when it is remembered that the chief limiting factor upon the bacteria

in the soil is the food supply. In liquid cultures, on the other
hand, the limiting factor is not the food supply but the accumu-
lation of detrimental by-products; the number of bacteria soon
reaches its maximum and then begins to decline gradually.
It can readily be seen that if predatory protozoa are added to
liquid cultures, in which the bacterial flora is in a comparatively
inactive condition due to the presence of harmful by-products,
a very striking reduction in bacterial numbers will be noted.
Whatever the effect of protozoa on bacteria in solutions may be
the results herein reported appear to indicate that under ordi-
nary conditions they are not able to limit the bacterial flora
when acting in soil.

Summary

1. Determinations made by means of the dilution method
indicate that the normal fertile soil has a protozoan content
approximating 10,000 per gram.

2. In the soils studied the flagellates were the predominating
type of protozoa and not the ciliates nor amoebae.

3. *Colpoda cucullus* appears to be the most widely distributed
ciliate in soil and is occasionally found in numbers approxi-
mating 1,000 per gram.

4. Certain of the soil flagellates are active in soils of normal,
and even subnormal, moisture contents.

5. Tests made with the ciliates *Colpoda cucullus*, *Balantio-
phorus elongatus* and *Oxytricha sp.* show that these organisms are
not active under ordinary soil conditions.

6. *Colpoda cucullus* is probably active whenever the moisture
content is much above normal, but not under ordinary conditions
of moisture.

7. Active soil protozoa attain greater numbers when inoculated
into previously sterilized soil than in normal soil.

8. Sterile soils when inoculated with normal soil and with
an artificial soil culture which is free of protozoa show a differ-
ence in the total number of bacteria as determined by the plate
culture method, due to a difference in the complexity of the two
flora.

9. A great difference may exist in the number of bacteria as determined by the plate culture method, due to a difference in the complexity of the flora, between soils which are free of protozoa.

10. Experiments with soils containing protozoa and free of protozoa showed that the bacterial flora in the two soils behaved in exactly the same way when exposed to different conditions of temperature and moisture content.

11. The data obtained indicate that soil does not contain a biological factor which is harmful to bacteria.

12. Pure culture tests with the ciliates, *Colpoda cucullus* and *Balantiophorus elongatus*, showed that these organisms are very detrimental to bacteria in solutions. In soil, since the ciliates are inactive, they are unable to affect the bacterial flora.

13. Pure culture tests with four types of active soil flagellates showed that these organisms were not capable of limiting the number of bacteria when acting in soil. One of the cultures, however, had a very marked limiting action upon the bacteria when tested in soil extract.

14. Treatment of soil with the ordinary amounts of volatile antiseptics (1 to 2 per cent) does not appear to simplify the protozoan fauna. A complex mixture of ciliates, flagellates and amoebae is to be found in cultures made from soils partially sterilized with volatile antiseptics.

15. As much as 10 per cent of carbon bisulphide and toluene when added to soil fails to exterminate the protozoa entirely.

16. The active soil protozoa which are at first suppressed by treatment with volatile antiseptics soon begin to multiply so that they are again found in numbers equal to those of untreated soil within one month after treatment.

17. The maximum number of bacteria in partially sterilized soil is not found while the protozoa are suppressed but after they have again returned to their normal level. It appears that the development of these two classes of micro-organisms subsequent to treatment with volatile antiseptics runs parallel.

18. The reinoculation of partially sterilized soils with 1 per cent of normal soil fails to decrease the number of bacteria.

19. The treatment of soil with carbon bisulphide at 37°C. gives a very marked increase in the number of bacteria in the soils treated.

20. Sterilized soils which are reinoculated with normal soil and with partially sterilized soil show no essential difference in the numbers of bacteria which develop.

21. When volatile antiseptics are applied to sterilized soils reinoculated with and without protozoa no difference is to be noted between the behavior of the bacteria in the different soils.

22. No evidence has been obtained which indicates that the beneficial effect of partial sterilization is due to the elimination of a biological factor which is harmful to the bacteria.

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ARE SPORE-FORMING BACTERIA OF ANY SIGNIFI-CANCE IN SOIL UNDER NORMAL CONDITIONS?[1]

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Among the best known of the soil microörganisms are the spore-forming bacteria. They have been described as soil-bacteria ever since the first bacteriological investigations of soil were made; and a more thorough taxonomic study has been made of them than of any other bacteria except those which have sanitary significance. It is seldom, however, that they comprise more than 10 per cent of the total flora of soil. Hiltner and Störmer (1903)[2] recognized three groups of colonies upon gelatin plates made from soil: liquefiers, non-liquefiers and *Streptothrix* forms. The liquefiers averaged about 5 per cent of the total flora. The ordinary spore-forming bacteria in soil are all rapid liquefiers and must have been included in this 5 per cent mentioned by Hiltner and Störmer. Similar results have been obtained by various other investigators.

The spore-forming bacteria, *B. mycoides, B. cereus*, and *B. megatherium*, are practically always present in soil and have always been considered characteristic and important soil organisms. These bacteria develop on gelatin or agar plates much more rapidly than those which comprise the other 90 to 95 per cent of the soil flora, and form large, striking colonies. They are among the largest of all bacteria and have an unusually interesting morphology, so it is not surprising that they have been studied most extensively of all the soil organisms growing

on ordinary media, in spite of the fact that they are not very abundant in soil. In nitrogenous culture media these bacteria grow rapidly and cause a vigorous amonification. For this reason they have been assumed to be the important ammonifiers of the soil.

This assumption was accepted as reasonable when I began to study the bacteria of soil. The first suspicion to the contrary came when it was noticed that the numbers of these spore-formers in the soil remained almost constant under all conditions, while the other bacteria varied in number according to the moisture content, aeration of the soil, or other conditions. The most natural explanation for this seemed to be that these bacteria lived over unfavorable conditions in the form of spores. It was soon realized, however, that this argument could not be carried to its logical conclusion without assuming that spore-formers were normally present in soil only as spores; in which case naturally their numbers would not vary.

A series of tests to investigate this matter has been made at the New York Experiment Station during the past year. The method used depended upon the fact that spores can resist higher temperatures than the vegetative forms. To determine the number of spores and vegetative rods present in any soil, one lot of diluted soil-infusion was plated in the ordinary manner, while a parallel lot of the diluted infusion was heated before plating for fifteen or twenty minutes at 75 to 85°. Then the colonies of the three spore-bearers, *B. mycoïdes*, *B. cereus* and *B. megatherium*, appearing on each set of plates, were counted. The colonies that developed from the heated infusion were assumed to arise from spores only; while in the case of the unheated infusion colonies might arise from vegetative rods as well.

The culture medium used in these tests was gelatin.[3] On this medium each of the three organisms investigated produced a fairly characteristic colony, so that it was ordinarily possible to distinguish them with little difficulty from non-spore-formers on the plates made from unheated infusion. Plates were in-

[3] Twelve per cent of Gold Label gelatine dissolved in tap-water and clarified with white of egg.

cubated at 18°C. for seven days. This length of incubation was necessary in order to allow the late colonies (particularly of *B. megatherium*) to appear. The chief disadvantage of such a long incubation was that *B. mycoides* and *B. cereus* often had time to liquefy the plate completely unless high dilutions were used. Dilutions of 1–20,000 and sometimes even 1–100,000 or 1–200,000 were necessary in order to avoid this trouble. At such dilutions the numbers of colonies of the spore-forming bacteria were so few that a long series of plates had to be made in order to obtain a reliable count; and even then no significance could be attached to variations in the count unless they were quite large.

In the first of these tests a temperature of 85° was used; but later it was learned that at temperatures only about 10° higher than this large numbers of the spores were killed and it was suspected that even 85° might destroy some of them. For this reason 80° was used instead for a while, and in the last tests 75° was used. To test the efficiency of this last temperature the bacteria developing on the plates after heating the infusion were studied, and it was found that nothing but spore-bearing bacteria had survived (leaving out of account an occasional colony of some non-spore-forming type that might easily be due to air contamination).

The greatest source of error in this method, which could not well be avoided, is the possibility that the bacteria investigated may occur in soil in clumps or chains instead of as isolated individuals. There is some evidence that clumps of bacterial spores can be broken up by the action of heat, which would tend to increase the count in the heated infusion provided clumps do occur in the soil. No increase of any appreciable amount has ever been observed, however; and indeed, so far as microscopical examinations of soil have been made, no evidence has been obtained of chains or clumps of organisms of this type. For this reason this possibility of error did not seem great enough to invalidate the conclusions.

A series of twenty-six tests was made. The results are given in the following table. The most striking fact to be observed

TABLE I

Number of spore-forming bacteria in soil infusion, before and after heating. Determined by means of gelatin plates

DATE	TYPE OF SOIL,* AND STATE OF CULTIVATION	TEMPERATURE USED	NUMBER OF B. MYCOIDES, B. CEREUS AND B. MEGATHERIUM PER GRAM OF SOIL		
			In unheated soil-infusion	In heated soil-infusion	Difference in favor of the unheated soil-infusion
1915					
Jan. 23	Dunkirk silty clay loam. Potted. Fallow.	85°	900,000	370,000	+ 530,000
Feb. 10	Dunkirk silty clay loam. Cultivated.	85°	850,000	600,000	+ 250,000
Feb. 19	Dunkirk silty clay loam. Potted. Fallow.	85°	900,000	430,000	+ 470,000
Feb. 25	Dunkirk silty clay loam. Sod.	85°	700,000	875,000	− 175,000
Mar. 10	Dunkirk silty clay loam. Sod.	85°	425,000	40,000	+ 385,000
Mar. 11	Dunkirk silty clay loam. Sod. Sub-soil.	85°	625,000	550,000	+ 75,000
Mar. 13	Volusia silt loam. Cultivated.	85°	550,000	275,000	+ 275,000
Sept. 16	Dunkirk silty clay loam. Sod.	85°	400,000	425,000	− 25,000
Sept. 21	Dunkirk fine sand. Sod.	80°	725,000	680,000	+ 45,000
Sept. 21	Dunkirk fine sand. Cultivated.	80°	250,000	625,000	− 375,000
Sept. 24	Ontario fine sandy loam. Sod.	80°	1,100,000	900,000	+ 200,000
Sept. 24	Ontario fine sandy loam. Cultivated.	80°	975,000	1,100,000	− 125,000
Oct. 19	Dunkirk silty clay loam. Freshly potted.	80°	525,000	400,000	+ 125,000
Oct. 20	Honeoye stony loam. Sod.	75°	800,000	1,150,000	− 350,000
Oct. 20	Honeoye stony loam. Cultivated.	75°	750,000	1,200,000	− 450,000
Oct. 22	Dunkirk gravelly loam. Sod.	75°	425,000	550,000	− 125,000
Oct. 22	Dunkirk gravelly loam. Cultivated.	75°	1,500,000	1,450,000	+ 50,000
Nov. 9	Dunkirk silty clay loam. Potted.	75°	700,000	1,050,000	− 350,000
Dec. 7	Dunkirk silty clay loam. Potted.	75°	800,000	650,000	+ 150,000

Dec. 7	Dunkirk silty clay loam. Dried. Potted	75°	775,000	650,000	+ 125,000
Dec. 7	Dunkirk silty clay loam. Manured. Potted	75°	1,350,000	1,050,000	+ 300,000
Dec. 7	Dunkirk silty clay loam. Manured and dried. Potted	75°	1,050,000	1,000,000	+ 50,000
Dec. 25	Dunkirk silty clay loam. Potted	75°	925,000	525,000	+ 400,000
Dec. 25	Dunkirk silty clay loam. Dried. Potted	75°	550,000	200,000	+ 350,000
Dec. 25	Dunkirk silty clay loam. Manured. Potted	75°	1,600,000	1,450,000	+ 150,000
Dec. 25	Dunkirk silty clay loam. Manured and dried. Potted	75°	400,000	375,000	+ 25,000
	Average of first eight tests	85°	670,000	445,000	+ 230,000
	Average of ninth to thirteenth test, inclusive	80°	715,000	740,000	− 26,000
	Average of last thirteen tests	75°	894,000	869,000	+ 21,000
	Average of last eighteen tests	80°–75°	844,000	833,000	+ 5,300
	Average of all twenty-six tests		788,000	712,000	+ 76,000

*The soil nomenclature of the Bureau of Soils is used in this table. The soils mentioned are described in the Soil Survey of Ontario County, New York, published by this Bureau.

at first glance is the regularity of the numbers of these organisms in the unheated infusion. The highest count is 1,500,000 and the lowest 400,000. Compared with bacterial counts in general, these show remarkable regularity, especially when it is considered that the soils varied from poor sand to richly manured loam and that the counts were made on plates of such high dilution that comparatively few colonies were obtained on each plate. The counts obtained from the heated infusion are not quite as regular; but if the first eight tests are excluded—in which the use of 85° may have killed a few spores—there is scarcely any more variation than in the case of the unheated infusion.

Because of this regularity in the counts it is possible to obtain general averages that can be fairly compared with each other. The average count from the unheated infusion is 788,000, from the heated infusion 712,000. This slight difference indicates that there are very few if any of the organisms present in soil in a form that can be killed by the temperatures used. Studying the figures more closely it will be noticed that the greatest differences between the two counts occurred in the first eight tests, in which 85° was used. The average count in these first eight tests, unheated, was 670,000, while the average count, heated, was 445,000. In the last eighteen tests, however, both counts averaged nearly the name, 844,000 and 833,000, respectively.

A more careful analysis of the data yields similar results. The last column of the table shows the difference between the two counts with a plus sign before it if the count obtained from the unheated infusion was the higher, with a minus sign if that from the heated infusion was the higher. It will be seen that there are eighteen cases in which the plus sign is used, and in these cases the greatest difference was 530,000, or if the tests are excluded in which 85° was used, it is 400,000. On the other hand in the eight tests in which a greater count was obtained from the heated infusion there is one difference as large as 400,000. The average difference between the two counts is 76,000 in favor of the unheated infusion; while if the

first eight tests are excluded it is only 5,300, an almost negligible figure.

It might be concluded from these data that some vegetative forms do exist in normal soil and for this reaon a higher count was obtained eighteen times from the unheated infusion; while in the other eight cases a higher count was obtained from the heated infusion because clumps were broken up by the heat. It is improbable, however, that these two factors should ordinarily so nearly neutralize each other; nor is it possible, if this explanation is used, to account for the greater average difference in the first eight tests than in those in which lower temperatures were used. It seems more reasonable to explain most of the differences in either direction as lying within the experimental error—a perfectly plausible assumption in view of the high dilutions used;— or if this is not enough to explain all the cases in which the higher count was obtained from the unheated infusion, to assume that an occasional less resistant spore was killed by the temperatures employed. The evidence all seems to indicate that the three organisms investigated do not occur in soil under normal conditions as active vegetative forms, but as spores. It is true that there are other spore-forming bacteria in soil besides these three types, in regard to which definite data could not be obtained because their colonies are not characteristic enough to be recognized with certainty; but none of them are as constantly present as the three types studied, and what evidence is at hand suggests that the same facts are true in regard to them as in regard to *B. mycoïdes*, *B. cereus* and *B. megatherium*.

It stands to reason, however, that these bacteria, so universally present in soil, must grow and multiply under some natural conditions. It is known that they ordinarily thrive in the presence of organic matter; so it seemed not improbable that they would multiply if manure were added to soil. A single experiment has been undertaken to test out this point, but with negative results. In a pot of soil, mixed with a heavy application of fresh horse manure, kept under observation for two months, there was at first a very great increase in the number of non-

spore-bearers, but no appreciable multiplication of spore-formers; nor was there any decrease large enough to be detected in the number of actual spores. Meanwhile the odor of the soil was enough to show that ammonification was vigorous. It is perfectly possible that a repetition of this test might yield different results; but evidently this experiment did not furnish the right conditions for the growth of the spore-forming bacteria. Also it is plain that ammonification can take place without them.

These results leave our knowledge as to the significance of spore-forming soil bacteria in a rather unsettled state. It has been quite generally taken for granted in the past that they are active in soil and of great importance. Perhaps their striking appearance in plate culture has led to the assumption that they could grow equally vigorously in soil. Yet they comprise but a small part of the soil flora, and even at that they do not seem to be present in vegetative form under normal conditions. Spores are generally regarded as inert.

Never the less these spore-forming bacteria of the soil do not decrease in numbers, and spores cannot live forever. Their occurrence in soil cannot be due to accidental contamination, or their numbers would not be so constant. If it is true, as these results indicate, that they are of practically no importance under normal field conditions, it becomes a matter of much interest to learn under what conditions they can become active and multiply.

SUMMARY

1. The number of spore-forming bacteria in soil is relatively constant and is about the same in all the soils studied. Three of the spore-forming bacteria aways present in soil—*B. mycoides*, *B. cereus*, and *B. megatherium*—were selected for the purpose of comparison, because their colonies on gelatin plates are quite readily distinguishable. The total number of these three organisms, as determined by means of gelatin plates, proved to be between 400,000 and 1,500,000 per gram in the soils studied. They always comprised less than 10 per cent and usually less than 5 per cent of all the colonies developing on gelatin.

2. When soil-infusion was heated before plating at a temperature (75–85°C.) high enough to kill the vegetative forms of bacteria, nearly if not quite as many colonies of these spore-forming bacteria developed as when it was plated unheated. In about one-third of the cases, indeed, their numbers were actually slightly higher on the plates made after heating; although all such differences undoubtedly lay within the limits of the experimental error. This suggests that these bacteria occur in normal soil as spores rather than in a vegetative state.

3. No increase in the total number of these organisms nor decrease in the number of their spores could be detected in a pot of soil to which fresh manure had been added.

4. These results throw considerable doubt on the common assumption that these organisms are important ammonifiers in the soil. They raise the question as to what possible soil conditions favor their growth and multiplication.

A POSSIBLE FUNCTION OF ACTINOMYCETES IN SOIL[1]

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It is not generally agreed whether *Actinomycetes* are to be classed with bacteria or with molds. They are thought to belong with the *Hyphomycetes* by some mycologists; but those that occur in the soil have generally been considered in connection with the bacterial flora rather than with the soil fungi. The reason why they have been studied by soil bacteriologists may be partly because *Actinomycetes* can be handled by much the same methods as the lower bacteria; and partly because both of these groups seem to be much more numerous than molds proper in normal soil.

The abundance of *Actinomycetes* in soil has been recognized for some time. In 1903 Hiltner and Störmer (1903) showed that of the colonies developing on gelatin plates from normal soil, 5 per cent were ordinarily liquefiers, 70 per cent nonliquefiers, and 20 per cent *Streptothrix* forms (a name often, although incorrectly, applied to this group). Probably everyone who has plated soil in gelatin, provided he has incubated his plates long enough for the slow-growing organisms to appear, will recognize these figures as typical of ordinary soil.

Perhaps the most interesting recent work on soil *Actinomycetes* is that of Krainsky (1914). It contains a valuable classification of these organisms and shows that the reason why few species have been distinctly recognized in the past is because the *Actinomycetes* require special media in order to bring out their specific characteristics. His further contention, however, that these special media are necessary in order to show the abundance of

Actinomycetes in soil is not correct. With his special media he claims to have found as many as 20,000 per gram of soil; but he overlooks the fact that Hiltner and Störmer (1903) found as many as 2.5 millions per gram. Moreover, in the work that forms the basis of the present paper, 2 or 3 million per gram has proved to be a very common figure; while on certain occasions the number has reached 12 to 14 millions. Occasionally over half the colonies developing on gelatin have been *Actinomycetes* —this in spite of the fact that Krainsky claims their growth to be suppressed by ordinary media.

The great abundance of *Actinomycetes* in soil has led to many speculations as to their significance. It has often been stated that they are active agents in the decomposition of organic matter; but their part in this process has not been definitely studied. Beijerinck (1900) showed that one type was often present in the corky layer of various roots. He called this type *Streptothrix chromogena* after Gasperini (1894) (who, however, had called it *Actinomyces chromogenus*). This type is one of the most numerous in soil; yet in the light of recent work it must be regarded as a group rather than a species. To this group belongs the causal organism of potato scab. Lutman and Cunningham (1914), indeed, have recently attempted to show that the cause of this disease must be renamed *Actinomyces chromogenus* because it agrees in every particular with Gasperini's description of that organism. This is plainly impossible; for Krainsky (1914) has shown that at least four separate species agree with the descriptions that have been given to *A. chromogenus*.

This fact brought out by Krainsky is very evident to anyone who uses his methods for studying the group. In fact it has proved possible, by the use of other special media[2] besides those described by him, to recognize many more types than those listed in his article. Work is now in progress along this line.

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[2] The medium which has given the best results of any yet investigated contains: 1000 cc. water, 15 g. agar, 10 g. glycerin, 1 g. sodium asparaginate, 1 g. glucose, 1.5 g. $NH_4H_2PO_4$, 0.2 g., $MgSO_4$, 0.1 g. $CaCl_2$, 0.1 g. KCl, trace $FeCl_3$. Further media are now being tested out that may prove even more satisfactory.

This complexity in the group and the confusion in nomenclature, however, must not hide the fact that an *Actinomyces* causes potato scab, nor that Beijerinck, approaching the subject from an entirely different angle, has shown them to be associated with the roots of other plants. It is also to be remembered that *Actinomycetes* are thought to be concerned in the decomposition of organic matter. Some recent observations at the New York Experiment Station bear on this point.

In the course of a qualitative study of the bacteria in certain New York State soils, it was early recognized that there was a great similarity between different soils in the relative numbers of *Actinomycetes* and lower bacteria present, provided the soils were in the same state of cultivation. Later it also became evident that the *Actinomycetes* were practically always present in greater abundance in old sod soil than in soil recently cultivated. This difference is shown in Table I, in which the numbers of *Actinomycetes* found in 20 samples of various sod soils are compared with the numbers occurring in an equal number of samples of cultivated soil. Although it is possible to pick out numerous cases in which the number occurring in some one of the cultivated samples is greater than in some of the sod samples, nevertheless the average number in sod soil is twice that in the cultivated soil. The table also shows that the *Actinomycetes* averaged 39.4 per cent of the total flora of sod soil, but only 21.3 per cent of the flora of cultivated soil. There is only one instance (October 22, 1913) in which the percentage of these organisms in sod soil is as low as their average percentage in cultivated soil, and only one (January 4, 1911) in which their percentage in cultivated soil is as high as their average percentage in sod soil.

These figures furnish a strong indication that *Actinomycetes* are more numerous in sod than in cultivated soil; but even before all the data given in Table I were collected the importance of making a more satisfactory comparison was realized. To do this, a study was made of a considerable variety of soil types,[3]

[3] The soil nomenclature of the Bureau of Soils has been used in this work. The soils mentioned are described in the Soil Surveys of Ontario and Tompkins Counties, New York, published by this Bureau.

TABLE I

Number of Actinomycetes in miscellaneous samples of sod and cultivated soils. Determined by means of gelatin plates

	SOD SOIL				CULTIVATED SOIL		
Date	Soil type	Number per gram	Per cent of total flora	Date	Soil type	Number per gram	Per cent of total flora
Nov. 8, 1911	Dunkirk fine sand	1,000,000	28.0	Feb. 7, 1910	Dunkirk clay loam	4,000,000	18.0
Apr. 29, 1913	Dunkirk fine sand	3,800,000	38.0	May 28, 1910	Dunkirk clay loam	2,000,000	29.0
June 4, 1913	Dunkirk sandy loam	2,800,000	36.5	Aug. 20, 1910	Dunkirk clay loam	2,000,000	23.0
Oct. 27, 1913	Muck	33,000,000	28.0	Jan. 4, 1911	Dunkirk clay loam	6,000,000	40.0
Apr. 24, 1913	Ontario fine sandy loam	8,000,000	36.5	Feb. 8, 1911	Dunkirk clay loam	4,800,000	35.0
May 7, 1913	Loam. Type not known	3,500,000	35.0	Nov. 24, 1911	Dunkirk silty clay loam	6,300,000	21.5
May 12, 1913	Honeoye stony loam	8,000,000	43.0	Jan. 13, 1912	Dunkirk silty clay loam	6,000,000	17.5
May 14, 1913	Honeoye stony loam	8,500,000	30.0	Jan. 23, 1912	Dunkirk silty clay loam	2,800,000	25.0
May 26, 1913	Dunkirk fine sandy loam	7,000,000	56.5	Apr. 24, 1912	Dunkirk silty clay loam	3,800,000	16.5
June 29, 1913	Ontario loam	7,500,000	34.5	Apr. 24, 1912	Volusia silt loam	2,200,000	11.5
July 11, 1913	Heavy loam. Type not known	12,800,000	38.5	June 5, 1912	Dunkirk silty clay loam	3,200,000	16.0
Sept. 15, 1913	Dunkirk loam	4,200,000	54.0	June 5, 1912	Volusia silt loam	2,600,000	15.5
Sept. 29, 1913	Genesee silty clay loam	9,000,000	75.0	June 20, 1912	Volusia silt loam	3,200,000	23.5
Oct. 22, 1913	Genesee loam	9,500,000	21.5	Sept. 7, 1912	Volusia silt loam	4,000,000	26.6
Nov. 12, 1914	Dunkirk silty clay loam	6,000,000	42.8	Oct. 11, 1912	Volusia silt loam	1,200,000	11.0
Nov. 25, 1914	Dunkirk silty clay loam	8,300,000	37.8	Sept. 28, 1912	Dunkirk silty clay loam	8,200,000	21.0
Nov. 27, 1914	Dunkirk silty clay loam	4,500,000	45.0	Oct. 25, 1912	Dunkirk silty clay loam	3,300,000	17.5
Feb. 25, 1915	Dunkirk silty clay loam	10,500,000	23.8	Dec. 3, 1912	Dunkirk silty clay loam	5,300,000	15.2
Mar. 10, 1915	Dunkirk silty clay loam	8,700,000	43.7	July 0, 1913	Dunkirk silty clay loam	2,600,000	13.6
Mar. 11, 1915	Dunkirk silty clay loam	8,000,000	39.0	Aug. 5, 1914	Volusia silt loam	3,000,000	30.0
Average		*7,750,000	39.4	Average		3,800,000	21.3

* The first four counts in this column are omitted from the average. The total numbers of bacteria per gram are so low in sand and so high in muck that they cannot fairly be included with the rest.

and in order to avoid as many as possible of the other factors that might be involved in a comparison of miscellaneous soils two samples were always collected on the same date, from spots in the same soil not more than a few yards apart, one in old sod, the other in a cultivated field. In this series of tests thirty-eight pairs of samples were taken. Also a second shorter series of tests was made to compare the *Actinomyces* flora of three neighboring spots in a single soil type (Dunkirk silty clay loam), one spot in fallow soil, one in old sod and the third in a field which had been in grass for two or three years only.

All of the counts in these tests were made by means of gelatin plates, because in the earlier work gelatin had been found the best of the various media used for distinguishing *Actinomyces* colonies from those of the lower bacteria. The gelatin used sometimes contained soil-extract and sometimes tap-water alone.[4] Plates were always incubated for seven days at 18°C. before counting.

The results of the first series of tests are given in Table II. It will be seen that the average number of *Actinomycetes* in sod soil is nearly twice as high as the average number in cultivated soil and that they averaged 37.5 per cent of the total flora in sod soil but only 20.5 per cent of the flora of cultivated soil. These general averages are much like those given in Table I, but they tell only a part of the story, as it is possible for individual exceptions to obscure the differences in the average. In order to show the differences more plainly, the individual ratios were determined and averaged. In the sixth column of Table II is given the ratio of the actual number of *Actinomycetes* in the sod soil to the number in the corresponding samples of cultivated soil; in the last column of the table is given the ratio of the percentage of *Actinomycetes* in sod soil to the percentage in the corresponding samples of cultivated soil. A study of these ratios brings out some information not shown by the general averages.

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[4] For the composition of these media see: Conn, H. J. Culture Media for Use in the Plate Method of Counting Soil Bacteria. N. Y. Agric. Exper. ta. Tech. Bul. 38, 1914.

TABLE II

Number of Actinomycetes in neighboring sod and cultivated spots of various soil types. Determined by means of gelatin plates

DATE	SOIL TYPE	ACTUAL NUMBER PER GRAM				PER CENT OF TOTAL FLORA		
		Sod	Cultivated	Difference	Ratio	Sod	Culti-vated	Ratio
1913								
Nov. 18	Dunkirk fine sand........	3,600,000	3,000,000	600,000	1.2 :1	45.0	33.0	1.4: 1
Nov. 20	Ontario fine sandy loam..	10,000,000	4,000,000	6,000,000	2.5 :1	37.0	19.0	1.9: 1
Dec. 1	Dunkirk fine sand........	2,700,000	1,600,000	1,100,000	1.7 :1	29.8	19.5	1.5: 1
Dec. 5	Ontario fine sandy loam..	6,300,000	5,300,000	1,000,000	1.2 :1	35.2	21.8	1.6: 1
1914								
Jan. 19	Ontario fine sandy loam..	10,000,000	7,000,000	3,000,000	1.4 :1	37.0	31.3	1.2: 1
Feb. 27	Dunkirk fine sand........	9,000,000	2,400,000	6,600,000	3.8 :1	15.8	2.2	7.2: 1
Mar. 14	Dunkirk fine sand........	3,500,000	3,200,000	300,000	1.1 :1	35.7	12.2	2.9: 1
May 9	Dunkirk fine sand........	3,300,000	2,800,000	500,000	1.2 :1	33.0	22.5	1.5: 1
May 18	Ontario fine sandy loam..	6,400,000	5,800,000	600,000	1.1 :1	32.0	21.0	1.5: 1
May 19	Dunkirk sandy loam......	1,800,000	3,200,000	−1,400,000	1 :1.8	36.0	35.5	1: 1
May 21	Muck...................	11,000,000	10,000,000	1,000,000	1.1 :1	14.5	19.5	1:1.3
May 22	Honeoye stony loam......	3,200.000	2,800,000	400,000	1.1 :1	32.0	18.8	1.7: 1
May 28	Ontario fine sandy loam..	10,500,000	4,300,000	6,200,000	2.4 :1	36.8	20.0	1.8: 1
May 29	Dunkirk silty clay loam..	8,000,000	4,000,000	4,000,000	2 :1	40.0	19.0	2.1: 1
Aug 8	Ontario loam...........	6,000,000	6,400,000	−400,000	1 :1.1	28.5	25.5	1.1: 1
Aug. 27	Dunkirk silty clay loam..	9,600,000	1,500,000	8,100,000	6.4 :1	60.0	14.8	4: 1
Aug. 28	Ontario loam...........	10,300,000	9,000,000	1,300,000	1.1 :1	44.5	28.8	1.5: 1
Sept. 1	Dunkirk silty clay loam..	12,000,000	2,400,000	9,600,000	.5 :1	34.4	14.0	2.4: 1
Sept. 2	Dunkirk silty clay loam..	7,000,000	2,200,000	4,800,000	3.2 :1	58.0	19.0	3: 1
Sept. 10	Dunkirk silty clay loam..	8,500,000	2,500,000	6,000,000	3.4 :1	47.2	13.8	3.4: 1
Sept. 11	Ontario fine sandy loam..	4,500,000	1,600,000	2,900,000	2.8 :1	45.0	16.0	2.8: 1

Date	Soil							
Oct. 23	Dunkirk silty clay loam	8,500,000	3,000,000	5,500,000	2.8 :1	38.6	10.0	3.9:1
Oct. 30	Dunkirk silty clay loam	4,700,000	2,700,000	2,000,000	1.7 :1	42.8	15.9	2.7:1
1915								
Sept. 8	Dunkirk silty clay loam	7,500,000	4,500,000	3,000,000	1.7 :1	64.0	23.0	2.8:1
Sept. 16	Dunkirk silty clay loam	12,000,000	2,500,000	9,500,000	4.8 :1	48.5	12.5	3.9:1
Sept. 21	Dunkirk fine sand	1,600,000	1,300,000	300,000	1.2 :1	27.5	22.0	1.3:1
Sept. 24	Ontario fine sandy loam	7,000,000	4,600,000	2,400,000	1.5 :1	30.0	20.0	1.5:1
Oct. 11	Dunkirk sandy loam	2,200,000	630,000	1,600,000	3.5 :1	32.5	17.5	1.9:1
Oct. 20	Honeoye stony loam	8,000,000	5,000,000	3,000,000	1.6 :1	30.8	19.5	1.6:1
Oct. 22	Dunkirk gravelly loam	13,500,000	3,500,000	10,000,000	3.8 :1	46.0	17.0	2.7:1
Nov. 4	Dunkirk fine sand	2,800,000	2,800,000	0	1 :1	35.0	3.8	1:1
Nov. 4	Honeoye stony loam	6,000,000	6,300,000	−300,000	1:1.05	21.5	25.0	1:1.2
Nov. 4	Dunkirk sandy loam	1,300,000	2,400,000	−1,100,000	1:1.8	29.0	36.5	1:1.3
Nov. 4	Dunkirk gravelly loam	9,500,000	5,500,000	4,000,000	1.7 :1	46.0	18.5	2.5:1
1916								
Jan. 27	*Dunkirk fine sand	1,600,000	1,200,000	400,000	1.3 :1	45.0	30.0	1.5:1
Jan. 28	*Honeoye stony loam	6,000,000	5,400,000	1,200,000	1.2 :1	36.5	35.5	1:1
Jan. 28	*Dunkirk sandy loam	1,800,000	1,600,000	200,000	1.1 :1	60.0	52.0	1.2:1
Jan. 29	*Dunkirk gravelly loam	12,800,000	10,500,000	2,300,000	1.2 :1	51.0	26.5	1.9:1
Average		6,850,000	3,800,000	3,000,000	2.15:1	37.5	20.5	2.1:1

* The last four pairs of analyses were made from the same samples as those on Nov. 4, 1915. During the intervening twelve weeks they had been kept in the laboratory. Meanwhile the Dunkirk fine sand and sandy loam had become almost completely dry. As these later analyses did not represent normal field conditions, their results are omitted from the averages in this table.

In making a comparison of these ratios it seemed reasonable to assume that those falling between the limits of 1.2 : 1 and 1 : 1.2 were so near unity as to indicate no real difference in numbers between the sod and cultivated samples. In the sixth column, giving the ratios of the actual numbers per gram, sixteen cases are listed that fell within these limits. There were only two cases (May 19, 1914 and the third on November 4, 1915) when the numbers in cultivated soil were enough greater than in sod to give a ratio outside these limits; and of these the greatest ratio was only 1 : 1.8. There were nineteen cases, however, in which the numbers in sod were sufficiently greater than in cultivated soil to give a ratio exceding 1.2 : 1; and of them the maximum ratio was 6.4 : 1. The average ratio of all thirty-eight cases was 2.15 : 1, which is larger than the ratio between the general averages of columns three and four. The figures which show what percentage of the total flora consisted of *Actinomycetes* are somewhat more striking. The average ratio, it is true, (as shown in the last column of the table) was 2.1 : 1 or practically the same as the ratio between the actual numbers per gram; but there were only four cases that fell between the limits 1.2 : 1 and 1 : 1.2 and only two (May 21, 1914 and the third on November 4, 1915) when the numbers in cultivated soil were enough greater than in sod to give a ratio outside these limits. These two cases both showed a ratio of 1 : 1.3 which is hardly to be compared with the maximum ratio, 7.2 : 1, in favor of sod soil.

The conclusion to be drawn from this comparison is that the few exceptional cases in which there were more *Actinomycetes* in the cultivated soil are completely overbalanced by the numerous cases in which there were more in the sod soil. In some of the border-line cases, moreover, the number of lower bacteria was greater in the cultivated soil than in the corresponding sod sample, with the result that the percentage of *Actinomycetes* was sometimes greater in the sod sample even though the actual number was the same in both samples.

The last four cases in the table are of special interest because they were analyses of the same samples collected on November

4, 1915, made after keeping the samples in the laboratory twelve weeks. On the date of collection the ratio obtained in the case of one pair of samples was in favor of the cultivated soil, while in two of the others case it was nearly unity. At the time of the later analysis the ratios in these three cases were still all near unity, although none of them were actually in favor of cultivated soil.

The results of the other series of tests, comparing three neighboring spots in a single soil type, are given in Table III. The

TABLE III

Number of Actinomycetes in three neighboring spots of a single soil type. A comparison of old sod, new sod, and cultivated soil. Numbers determined by means of gelatin plates

DATE	ACTUAL NUMBER PER GRAM			PER CENT OF TOTAL FLORA		
	Old sod	New sod	Cultivated	Old sod	New sod	Culti-vated
May 29, 1914...	8,000,000		*4,000,000	40.0		*19.0
September 1, 1914...	12,000,000		*2,400,000	34.4		*14.0
September 5, 1914...		5,000,000	3,000,000		20.8	15.2
September 10, 1914...	8,500,000	7,500,000	2,500,000	47.2	21.7	13.8
October 23, 1914...	8,500,000	7,800,000	3,000,000	38.6	25.2	10.0
September 16, 1915...	12,000,000	6,600,000	2,500,000	48.5	23.0	12.5
Average............	9,800,000	6,600,000	2,900,000	41.7	23.6	14.1

* The first two samples of cultivated soil were taken from a different spot from the rest, although similar in kind of soil and in state of cultivation.

numbers obtained in this test were so constant that the few analyses mean as much as longer series of irregular results. The average number of *Actinomycetes* in the old sod was 9,800,000 per gram, in the new sod 6,600,000 and in the cultivated soil, 2,900,000; or in percentages, they averaged 41.7, 23.6 and 14.1 per cent, respectively, of the total flora in these three spots. The lowest count (of *Actinomycetes*) in old sod was higher than the highest in new sod, and the lowest in new sod higher than the highest in cultivated soil. These figures indicate that the number of *Actinomycetes* in sod soil increases as the age of the sod grows greater.

The interpretation of the figures hinges upon the question whether these organisms should be regarded as filametous fungi producing spores or as unicellular bacteria occurring in filaments. On ordinary culture media they exist as branched filaments that break up under certain conditions into short rods or coccus-like bodies, known as conidia because of their similarity to the conidia of molds in method of formation. When such cultures are plated, each colony ordinarily comes from one conidium or group of conidia. If they grow similarly in the soil and if the conidia are actually spores, an increase in the number of colonies on the plates may indicate merely an increase in spore-production. A few observations are at hand, however, to indicate that *Actinomycetes* occur in the soil not as filaments but as chains of short rods or cocci closely resembling ordinary bacteria. If this is the normal mode of growth in the soil and if these bodies are individuals instead of spores, an increase in the number of colonies on the plates may be regarded as more nearly representing a true increase in the number of the organisms in the soil.

Making the assumption that the latter condition actually exists in the soil, which seems justified so far as the facts are known, there are two explanations of the higher numbers observed in sod soil that seem sufficiently probable to be considered. One is that sod soil becomes more compact in time than cultivated soil and that poor aeration favors the *Actinomycetes* in some way, in spite of the fact that they ordinarily seem to like a good supply of oxygen. This explanation does not well fit the facts, however; for it has been found that sod soil, dug up and well aerated and then kept in a pile for three months, may still retain its high *Actinomyces* content. The other explanation which has been considered is that the *Actinomycetes* are active in the decomposition of grass roots or perhaps of plant roots in general. In view of the past observations as to the association between *Actinomycetes* and plant roots, this explanation seems worth bearing in mind. Experiments are now being carried on which are designed to show whether or not this is the true function of *Actinomycetes* in soil.

SUMMARY

1. In general more colonies of *Actinomycetes* develop on plates made from sod soil than on those from cultivated soil. The average ratio between their numbers in neighboring sod and cultivated spots in the same soil type is slightly over 2: 1. The maximum ratio is about 6: 1.

2. *Actinomycetes* average about 38 per cent of the total flora of sod soil, as determined by means of gelatin plates, but only about 20 per cent of the total flora of cultivated soil.

3. In a study of three neighboring spots in a single soil type it has been found that *Actinomyces* colonies not only appear in greater numbers from sod than from cultivated soil, but also in greater numbers from old sod than from sod only two or three years old.

4. This relation has been found to hold with very few exceptions In the isolated cases where more *Actinomyces* colonies have developed from a sample of cultivated soil than from the corresponding sample of sod soil, the ratio has never been greater than 1.8: 1.

5. Although the reason for this difference in numbers has not been learned, a probable explanation seems to be that *Actinomycetes* are active in the decomposition of grass roots.

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PRACTICAL OBSERVATIONS ON THE TITRATION AND ADJUSTMENT OF CULTURE MEDIA

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Any one who studies the methods given in the various textbooks for the titration and adjustment of culture media, must be struck by the lack of uniformity of opinion. Not only is the beginner in media preparation bewildered but even the more experienced worker may be led into error. The difficulty arises from the fact that the complex nature of the materials dealt with is by no means fully understood, even in the case of the most fundamental culture media. In addition to this the changes that occur under even slightly different conditions and treatment are most confusing. As a result each laboratory is compelled to adopt the methods best adapted to its work and requirements, and each laboratory makes changes in these methods as need arises.

The many requests constantly made for information regarding the methods employed in our laboratories for the titration and adjustment of both general and certain special culture media seem to indicate the need for a detailed account of such procedures.

In this paper we have tried to incorporate the practical information gained after a number of years of experience. In addition, we have described experiments carried on with a view of clearing up, in a systematic manner, certain points upon which little, if any, information is available.

The *Standard Method*[1] of titrating media for water and milk analyses was devised in an attempt to secure uniform preparations of media at all times so that comparable results might be obtained.

[1] Committee on Standard Methods, 1905, 1913).

The directions are as follows:

Phenolphthalein shall be the standard indicator in obtaining reaction of all media. Tumeric paper possesses similar properties and its use advised where phenolphthalein is not available. Titrations and adjustments of reactions shall be made as follows:

Put 5 cc. of media to be titrated in 45 cc. of distilled water. Boil briskly one minute. Add 1 cc. phenolphthalein solution (5 grams of commercial salt in one liter of 50 per cent alcohol.) Titrate while hot (preferably while boiling) with $\frac{N}{20}$ caustic soda. A faint but distinct pink marks the true end point. This distinct pink color may be described as a combination of 25 per cent of red (wave length approximately 658) with 75 per cent of white as shown by the disks of the color top, described under Records of Tints and Shades of Color, p. 10. (The Standard[2] color disks used in teaching optics may be used for this purpose.)

In practice, titration is continued until the pink color of alkaline phenolphthalein matches that of the fused disks. All reactions shall be expressed with reference to the phenolphthalein neutral point and be expressed in percentages of normal acid or alkaline solutions required to neutralize them.

One of the objects of this paper is to consider whether the desired results are actually obtained by the Standard method or by modifications of this method.

In our laboratory one modification, that is, titration of broth at room temperature (about 20°C.) and of agar at a temperature of about 30°C. has given good results for a number of years past.

These and other experiences have led us to investigate further the following subjects:

a. The effect of prolonged heating on meat infusions and beef extract solutions as shown by the titration curves of both adjusted and unadjusted portions. The results of boiling samples of media in the casserole for titration.

b. The adjustment of broth and agar, including the remelting of solid media.

[2] A small sized top and disks costing only a few cents may be obtained from Milton Bradley Educational Company, Springfield, Mass.

c. The reaction of peptone solutions and the effects upon them of prolonged heating.

d. The question of indicators with consideration of the significance and sensitiveness of their end points in different media—also the method of choosing the one most suitable with reference to the hydrogen electrode as a standard.

MEAT INFUSIONS

It is a well known fact that each time a medium is heated to the boiling point, or above it, the reaction changes and becomes more and more acid, depending on the length of time and the degree of heating.   On this subject Eyre (1915) says:

Meat extract [meat infusion] is acid in reaction owing to presence of acid phosphates of potassium and sodium; weak acids of the glycolic series and organic compounds in which an acid character predominates.

Owing to the nature of the substances from which it derives its reaction, the total acidity of meat extracts [infusion] can only be estimated accurately when the solution is at the boiling point.   Prolonged boiling [as in media preparation] causes it to undergo hydrolytic changes which increase the acidity.

He states further that meat extract [infusion] becomes stable in reaction after being heated at the boiling point for forty-five minutes so that no additional increase of acidity occurs on further heating.

To procure more definite data as to the effect of heat on the acidity of media the following work was carried out:

Preparation of the meat infusions. Chopped lean veal was soaked over night in tap[3] water in the proportion of one pound of meat to one liter of water.   It was then heated at 45° to 55° C. for one hour.   At this point it was brought to a boil.   Then

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[3] Weekly analyses of the Croton water supply shows it to contain a negligible amount of mineral matter (only about 40 parts per million, expressed as total hardness.)   Where the water supply is at all "hard," it is advisable to employ distilled water exclusively.

the material (meat and watery infusion) was divided into three lots:

B[4] 30 was kept at the boiling point 30 minutes.
B 60 was kept at the boiling point 60 minutes.
B 120 was kept at the boiling point 120 minutes.
(Volumes being made up by addition of tap water.)

After being boiled, each lot was strained through cheese-cloth, then filtered through paper (S. & S. "Falten" filter) and cotton (first moistened with cold water to hold back the fatty substances.)

Each lot was titrated[5] and then divided again into two parts and one series (B 30, B 60, B 120) was run in the autoclave at least six successive times, without the addition of soda.

The second series (B 30 C, B 60 C, B 120 C) was corrected with normal sodium hydroxid to 1 per cent acidity ($+1$) and then run in the autoclave with the other set and under the same conditions.

In all the tests made the autoclaving was done at a pressure between 15 and 17 pounds as indicated by a Bristol recording pressure gauge. The heating was carried on up to a total of eight hours and titrations performed at one-half hour intervals for the first four half-hours; then at one hour, two hour and three hour intervals.

After each autoclaving the six samples were titrated and the corrected series (B 30 C, B 60 C, etc.) was adjusted again when necessary to plus one ($+ 1$).

Method of titration. Freshly boiled and cooled distilled water was used for all titrations. A 5 cc. sample of meat infusion was drawn off by means of a 5 cc. pipette and added to 45 cc. of distilled water in a casserole to which 1 cc. of a 1 per cent[6] solution

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[4] Preliminary titrations on samples before boiling had been labelled "A." As these had no significance they are omitted in this article.

[5] See: "Method of Titration."

[6] These are also variations from the standard method of using 0.5 per cent solution of phenolphthalein and twentieth normal sodium hydroxid solution. They are, however, in accordance with the methods in use for years in this laboratory and to preserve uniformity they were adhered to.

of phenolphthalein had been added.   While stirring the mixture, deci-normal[6] soda solution from a burette was run in without any heating whatever.   The end point taken was the first delicate pink tinge, observable throughout, which did not disappear after stirring the solution—and should not disappear for at least one minute.   The figures were then recorded.   The casserole with the mixture was then set over the flame, brought to a boil and boiled one minute by the watch.

Outline of Experiments

Meat infusions First lot of veal

- B 30   = preliminary boiling of 30 min.
- B 60   = preliminary boiling of 60 min.
- B 120   = preliminary boiling of 120 min.

- B 30 C = preliminary boiling of 30 min.
- B 60 C = preliminary boiling of 60 min.
- B 120 C = preliminary boiling of 120 min.

Corrected to plus one according to *room temperature* titration after each autoclaving.

- B_1 60   = preliminary boiling of 60 min.
- B_1 120   = preliminary boiling of 120 min.

- B_1 60 C = preliminary boiling of 60 min.
- B_1 120 C = preliminary boiling of 120 min.

Corrected to plus one according to *boiling temperature* titration after the *first* autoclaving. No further adjustments were made because of error in adding too much soda.

Meat infusions Second lot of veal

- B_2 30   = preliminary boiling of 30 min.
- B_2 60   = preliminary boiling of 60 min.
- B_2 120   = preliminary boiling of 120 min.

- B_2 30 C = preliminary boiling of 30 min.
- B_2 60 C = preliminary boiling of 60 min.
- B_2 120 C = preliminary boiling of 120 min.

Corrected to plus one according to *boiling temperature* titration after each autoclaving.

Since the boiling had caused the faint pink color to disappear, the hot mixture was then promptly titrated again, the same end point being approximated[7] as closely as possible, and the figures recorded. The room temperature figure plus that obtained after boiling one minute gave a total which represents the boiling titration figure as recorded in the charts.

In the first lot of veal, (series B 30, B 60, B 120), the corrections were made to plus one at the room temperature figures. A second lot of veal infusions (B_2 30, B_2 60, B_2 120) were prepared in the same manner as above. The corrections in this lot were made to plus one at the boiling figures.

DESCRIPTION OF CHARTS

In charts 1 and 2 are shown the uncorrected portions of meat infusions titrated after successive heatings in the autoclave and plotted according to both the room temperature and the boiling temperature figures. It will be seen that in each lot of meat the room temperature titrations fall into one group and the boiling titrations into another; also that the boiling figures are the higher.

Chart 3 is a sample[8] chart showing not only the uncorrected portion of B 120 as given in Chart 1, but also the corrected portion, B 120 C. This portion was corrected to plus one ($+ 1$) according to the room temperature titration figures and readjusted to plus one after each autoclaving, as shown by the α line. The α' line shows the figures of this same material when boiled one minute in the casserole and titrated hot. This line is hypothetical and shows only the amount of soda that would have been needed had the boiling figures been used for adjustment to plus one.

The θ line shows the total amount of acidity produced in the corrected portion even after the addition of soda, according to the room temperature figures.

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[7] The difficulty of catching the first color change of phenolphthalein in hot solutions will be discussed under "Indicators."

[8] This chart is typical also of the B 30 and B 60 sets.

KEY TO CURVE NOTATION.		
LETTER	LINE	DENOTES
α	———— ————	= corrected at room temperature
α¹	———— ·—— ·—— ·—	= " " " (boiling figures)
β¹	——— ·—— ·—— ·—	= " " " boiling temperature
β	—— ·· —— ·· ——	= " " " (room temp. figures)
γ	———————	= uncorrected (room temperature)
γ¹	— — — — — — —	= " " (boiling temperature)
Θ	——— · ——— · ——— · —	= Total acidity, corrected at room temp.
Θ¹	——— ·· ——— ·· ——— ·· —	= " " " " boiling temp.

CHART 1
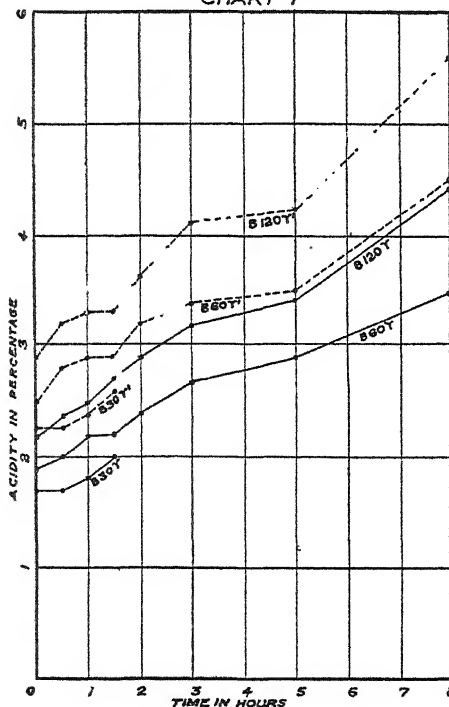
CHART 2
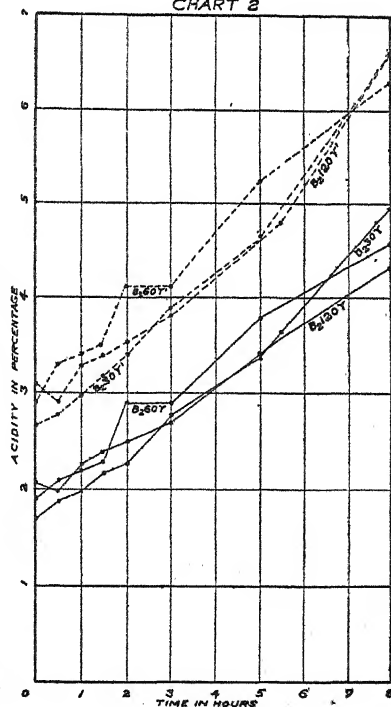
In Chart 4[9] is represented B_2 120 (second lot of veal) just as
B 120 is shown in Chart 3 except that here the corrected por-
tion (B_2 120 Cβ') was adjusted to plus one ($+1$) according to
the boiling titration figures. The β line shows the figures ob-
tained each time at room temperature before the sample was
boiled one minute in the casserole to give the boiling titration
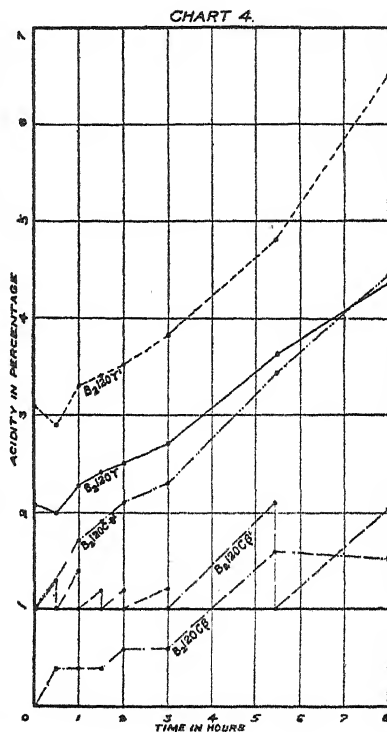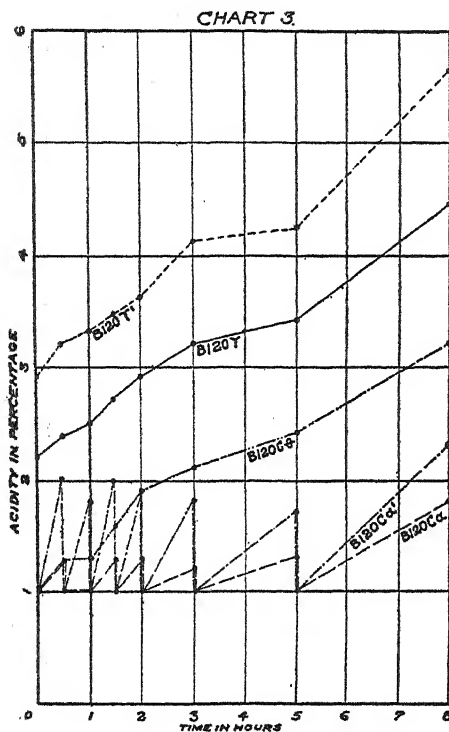
figures. The room temperature curve is inserted here for com-
parison.

The θ' line shows the total acidity produced in the portion
corrected according to the boiling titration figures.

In chart 5 all the total acidity lines of the various corrected
portions are compared. As with the uncorrected portions as

[9] This chart is typical also of the B_2 30 and $B_2$60 sets.

noted in charts 1 and 2, the total acidity lines fall into two groups according to the method of titration.

If chart 5, showing the total rises in acidity of the series B 30 C, B 60 C, B 120 C and the series B_2 30 C, B_2 60 C, B_2 120 C, were applied successively to the corresponding curves of the uncorrected series in charts 1 and 2, so that the point of origin in each set were the same, it would be seen that in every instance the

CHART 5.
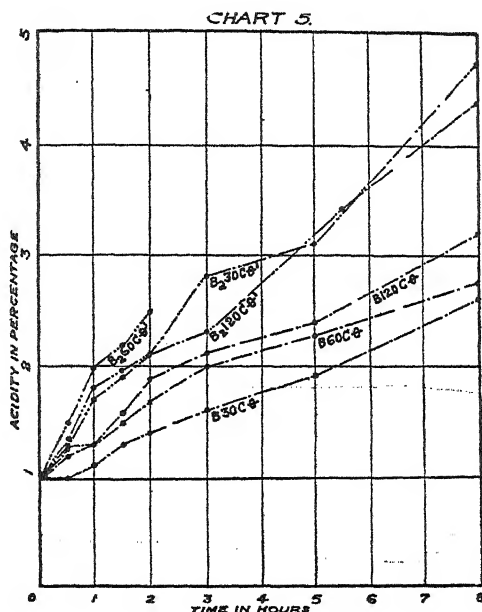
total rise of acidity of the corrected curve equals or exceeds that of the uncorrected portion.

From this it is plainly evident that, in spite of successive corrections of acidity with normal sodium hydroxid, hydrolysis not only continues on the application of heat but there is produced in meat infusion media approximately as much acidity as would be developed were no correction made.

Chart 6 shows the actual acidity of the different corrected portions after the successive adjustments and periods of heating. As can be seen, those portions adjusted according to the

CHART 6.

ACIDITY IN PERCENTAGE

TIME OF HEATING

½ HOUR   1 HOUR   1½ HOUR   2 HOURS   3 HOURS   5 HOURS   8 HOURS

KEY:

B30Cα   B60Cα   B120Cα   B30Cβ'   B60Cβ'   B120Cβ'

Corrected to 1.0% (Room temperature titration)
"   "   "   (Boiling   "   )

NOTE: #5 Broken after 3 hours.
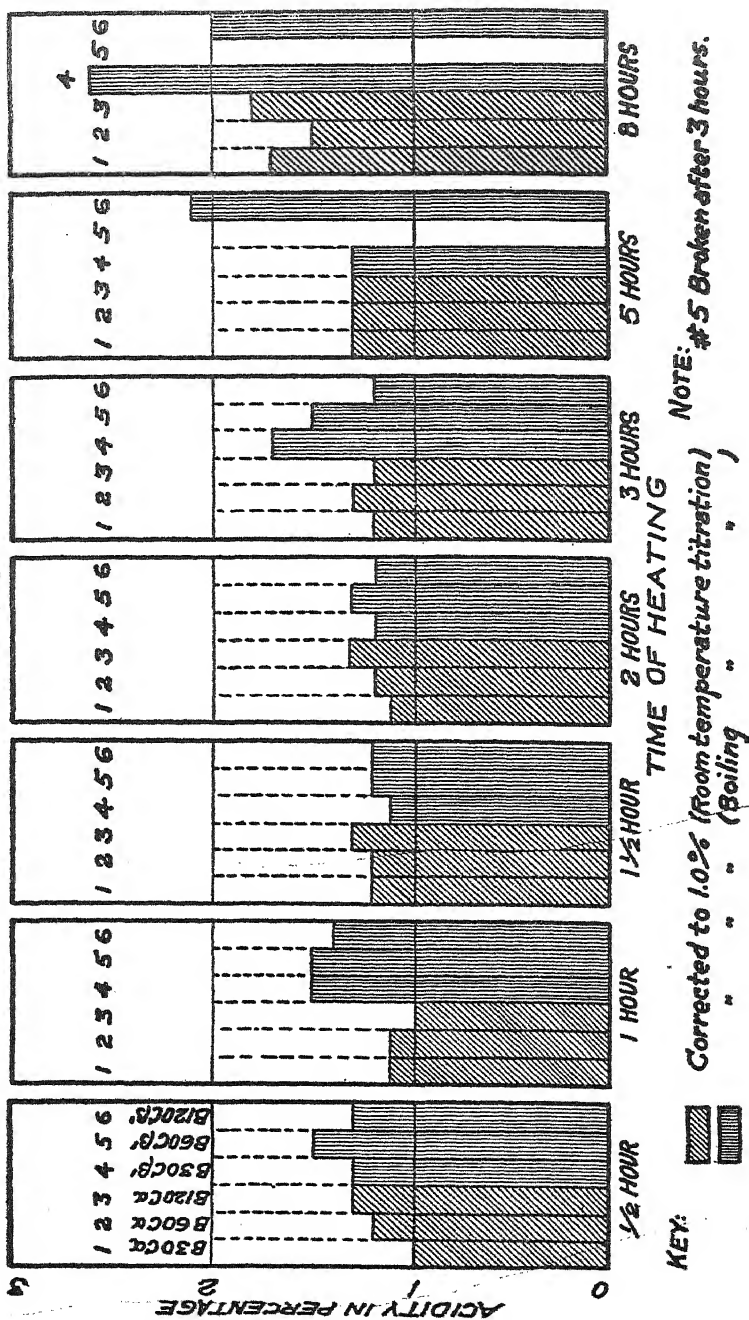
room temperature titration are in general nearer to the desired reaction of plus one ($+ 1$), especially in the first two half-hours—the periods of time of greatest practical interest.

In chart 7 are shown not only the uncorrected portions of B_1 60 (γ and γ') and B_1 120 (γ and γ') (duplicate material from

CHART 7.
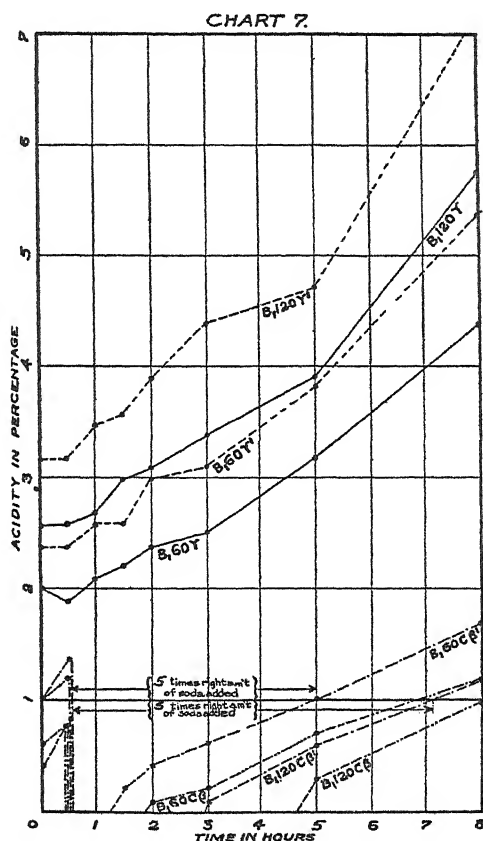
first lot of veal), but also the effect on the corrected portions of the addition of too much normal sodium hydroxid. By mistake after the first half hour in the autoclave and the subsequent titration, there was added to B_1 60 C β' approximately five times the amount of normal sodium hydroxid necessary to correct it to plus one, and to B_1 120 C β' also about five times

the right amount. These amounts were in accordance with the boiling titration figures in both cases. The quantities needed for correction were, in round numbers, twice as much for the latter as for the former. The relation of the quantities of alkali added may, therefore, be expressed by the numerical relation of ten to five. The portions of meat infusion (without further additions of soda) were then run as usual in the autoclave with the uncorrected portions and titrated at the same intervals.

Curiosity led us to continue these tests rather than discard them. In consequence, an interesting fact was brought out. To our surprise both over-corrected portions recovered the acidity of plus one (and more) but at different intervals of time. B_1 60 $C\beta'$ gained plus one at the end of the fifth hour while B_1 120 β' reached plus one at about the seventh hour.

In all the tests the curves show a distinct and steady rise in acidity. This rise is due to hydrolysis caused by heat and increases continually as more heat[10] is applied. Further, it is plainly evident that a preliminary heating to the boiling point for at least forty-five minutes as advocated by Eyre (1915) does not produce a stable reaction uninfluenced by further heating.

TABLE I

MEAT INFUSION	REACTION BEFORE AUTOCLAVING		AFTER 8 HOURS IN AUTOCLAVE		AFTER 14 HOURS IN AUTOCLAVE	
	R. T.*	B. T.†	R. T.	B. T.	R. T.	B. T.
$B_2$30................	1.7	2.7	4.9	6.5	7.4	10.5
$B_2$60................	1.9	2.9	4.6	6.3	7.3	10.4
$B_2$120................	2.1	3.1	4.3	6.5	6.9	9.3

*R. T. = room temperature titration.
*B. T. = boiling temperature titration.

In the above tests we have gone outside the limits of interest from the practical standpoint. We were led to this, however, in an attempt to locate the point of complete hydrolysis or maximum acidity of meat infusions. This goal was not reached,

[10] Three of the uncorrected portions, B_2 30, B_2 60, B_2 120 were run an additional six hours, making fourteen hours all told in the autoclave at 15 pounds pressure. The results are shown in last columns of Table I.

as stated above, even after fourteen hours autoclaving. We are continuing this work.

In the usual preparation of media from meat the total amount of heating in the autoclave varies from one half hour, at about 15 pounds pressure, for sterilization of ordinary broth, to two and one-half hours in the preparation and sterilization of agar.

It developed in the tests made on the corrected meat infusions that the change in acidity in the first half hour in the auto-clave (the usual time for the sterilization of finished media) varied from nothing to 0.3 per cent at the room temperature figures, while in boiling temperature figures the change in acidity was 0.3 to 0.4 per cent (see chart 6).

The nature of the acid products which are formed as a result of the hydrolytic decomposition on boiling with sodium hydroxid may be different from those produced by hydrolysis alone when boiling unadjusted media. Therefore, while the reaction may be adjusted in each to the same point of acidity, the behavior of the media toward the various organisms may not be the same.

Similar tests to those above were made on Liebig's beef extract dissolved in tap water, filtered and titrated in a similar fashion. These tests showed no change in the reaction of the uncorrected series even after eight hours heating. Although a beef extract solution is undoubtedly quite stable,[11] as compared with meat infusions, a sample corrected[12] to neutral, after two hours in the autoclave, rose 0.2 per cent in acidity. This was corrected to neutral once more and did not change again although heated four hours longer.

While the above change is almost negligible, the addition of peptone to this as to other media, raises the change in acidity further. This must be taken into consideration in the preparation of media with beef extract when delicate end points are desired.

[11] This stability is due probably to very prolonged heating in the preparation of the beef extract itself.

[12] Correction based on room temperature titration.

<center>BROTH[13]</center>

The tests on the meat infusions were carried out before any peptone or salt had been added. In the preparation of broth the choice of titration methods must of course be governed by the manner of preparing the meat juice in the preliminary steps. The relative merits of pressing out the meat juice before or after heating the soaked meat must be determined by experiment in the kinds of work for which the media are destined.

For those workers whose needs and experience lead them to express the meat juice in the cold state and then dissolve the peptone and salt with very little preliminary heating, the use of the boiling titration for correction is essential, in order to approximate the future conditions due to further heating and sterilization after the reaction of the batch of medium has been set.

In this laboratory the best toxin production has seemed to be obtained when the meat juice is pressed out after heating large amounts (20 liters) for one hour at 45° to 55°C. and then boiling up strongly until the meat coagulates. The meat infusion[14] is then strained through cheese-cloth. After the requisite amount of peptone and salt have been dissolved by further heating up to the boiling point and the mixture boiled one half hour, the reaction is set according to a room temperature titration.

If the specimen is boiled[15] in the casserole before titration, it no longer represents the lot of broth in the kettle but has risen somewhat in acidity. Consequently, if the large lot be adjusted according to the boiling titration a false correction is made. To be sure, after adding the normal soda solution,

[13] Broth = meat infusion plus peptone and, usually, salt.

[14] This method proves useful in other lines of work for at this point the infusion can be filtered, sterilized and stored. It is ready for further use on the addition of any suitable peptone and may be set at any desired reaction; or it is ready as a basis for making agar.

[15] At this point may be mentioned the length of time recommended for boiling by different authors. Heinemann (1911) heats "to boiling." Jordan (1914) MacNeal (1914) and the Standard Method (1913) boil one minute. Park and Williams (1914) boil two minutes, Abbott (1915), Abel (1914), Hiss and Zinsser (1914), Mallory and Wright (1915), Swithinbank and Newman (1903) boil for three minutes.

any further boiling of the large lot, together with the final sterilization raises the acidity but not to just the desired point as shown in the tests on meat infusions (chart 6). For example, the final reaction of + 1.2 in the case of diphtheria toxin broth, is found to be uniformly obtained by titrating at room temperature and setting the reaction to + 1. This allows 0.2 rise due to heating if the broth is to be sterilized at 15 pounds pressure (121.6°C.) for one half hour. If the sterilization is to be carried on at only 5 pounds pressure (108.8°C.) for one hour on three successive days, or in the Arnold sterilizer, streaming steam (100°C.), for the same length of time, the reaction is set at + 1.1 as the more moderate heating raises the broth only about 0.1 per cent in acidity, making the finished product + 1.2 in reaction. The use of this method for the diphtheria and tetanus toxin broths for a number of years has shown fully its value and it is the method still employed in our laboratory.

On the other hand Eyre (1915) states that the correct estimation of acidity present can be made only by titration at the boiling point. Judged from our results as shown in chart 4 by the β' and β curves, this statement is erroneous. The β' curve shows the reactions and successive adjustments to plus one (+ 1) based upon the *boiling temperature* figures. The reactions at room temperature of this material are shown by the β curve. This latter is far below the β' curve (from 0.5 to 1 per cent), and gives the actual reaction of the medium at a temperature nearer that of the incubator (37.5°C.)[16]

To make this point clear, let us assume for example that a medium is to be adjusted to a definite acidity of + 1, according to the boiling titration, as stated in the Standard Method. The real reaction at which the bacteria will then be grown in the incubator is not that indicated by the boiling titration figure but a reaction which is lower in acidity to an extent of about one per cent—in other words, almost neutral.

On the other hand in chart 3 (α' curve), there is shown a similar meat infusion, adjusted to plus (+ 1) at *room temperature*

[16] It is only after four hours' autoclaving that the room temperature reaction of plus one (+1) is reached.

titration. The boiling titration figures are plotted as curve
α'. The room temperature titration curve (α) comes so near
the desired reaction of plus one ($+1$) that even a mere glance
will suffice to convince one that the room temperature titration
approaches more closely the one per cent line, which is the acid-
ity we set out to secure.

This was not surprising to us as our practical experience for
several years past had indicated such a condition. The modifica-
tion, (page 212), devised at that time and now further sub-
stantiated by these experiments, has proved to be so very useful
in its results that it is employed in these laboratories for nearly
all the routine preparation of some fifty different kinds of media,
aggregating over 8000 liters per year.

STERILE SODA

In order to avoid the complications of further hydrolysis
and precipitation after the addition of soda to a medium which
must later be sterilized, it has been suggested that steriliza-
tion be done first and that sterile soda be carefully added after-
ward according to the titration of samples withdrawn under
sterile conditions. This has been practised by some workers,
apparently with success. So far, in our laboratory, it has
shown no advantages in the production of diphtheria toxin
broth. Further work in this line is contemplated.

AGAR

When the need arose of supplying large amounts of neutral
veal agar for the growing of the gonococcus, streptococcus and
other organisms in bulk for antigens, difficulty was experienced.
To grow these organisms in large lots with unfailing success
is not always easy. Our trouble seemed due chiefly to the
reaction of the medium. Finally the modified titration method
was adopted for agar also.

Since agar solidifies at a little below 40°C. the room temperature
titration was not suitable. At first any temperature between

40° and 50°C. was used.   This was reduced later to about 30°C.[17]
Five cubic centimeters of the hot agar are added by means of
a pipet to 45 cc. of distilled water—temperature about 30°C.
(verified with a thermometer).   One cubic centimeter of phenol-
phthalein (1 per cent solution in 50 per cent alcohol) is added
and the titration performed at once.

It is now our custom to titrate each batch of neutral veal
agar at least twice during its preparation making the necessary
adjustments of reaction.   Here, as with the broth, allowance
must be made for further heating in the autoclave.   Experience
shows that agar made from meat infusion rises in acidity usually
about 0.3 to 0.4 per cent. at 15 pounds pressure during one and
one-half to two hours.[18]   Therefore, 3 to 4 cc. of normal soda
should be added per liter in excess of the amount required to
secure the phenolphthalein neutral point at the time of the
first titration.   The second titration is made just after filtra-
tion, before tubing and sterilizing.   If the amount of soda needed
does not exceed 0.2 per cent, little if any precipitate occurs
on heating further.   If more than the above amount is needed
in adjusting the reaction, the medium should be heated in the
Arnold for half an hour and the precipitate filtered out, before
tubing and sterilizing.

In very careful work the medium is also titrated a third time
as it comes from the autoclave.   For the last test a tube or small
bottle of neutralized glassware should be used in order that the
reaction of the agar may be unaffected by its container.   This
sample is tested before it hardens—as remelting would raise its
acidity further.   On the addition of phenolphthalein, it should
show a very delicate shade of pink if it is "neutral."

REMELTING OF SOLID MEDIA

An important factor to be considered in the adjustment of
media is the remelting of solid media for the addition of sterile
substances such as blood, serum, etc., or for the purpose of

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[17] This slight difference of temperature had no noticeable effect on the results
of the titration.

[18] This time is necessary for melting (and clearing with egg) of large batches
of agar (5 to 12 liters).

immediate use in plating.   If, for example, the whole is to be neutral to phenolphthalein when entirely finished an over neutralization is necessary to allow for the acid changes during the re-heating, as in making Bordet Gengou medium.

Since, in spite of the addition of soda for the correction of a medium further hydrolysis occurs when heat is applied, especially in the autoclave, it is impossible to know the exact reaction a medium will have when sterilization is complete or when the medium is re-melted.   In practical work, however, it has been found that an over neutralization of 0.1 to 0.3 per cent has given good results when the titration is performed at 30°C.

The *re-sterilization* of media without suitable correction to allow for the effects of heating is to be avoided if a very definite end point is desired.

PEPTONES

The present necessity of finding substitutes for Witte's peptone, so long the standard in bacteriological work, has led us to test the reaction of various peptones on the market.   A 1 per cent solution of each in distilled water was boiled one minute and then filtered through cotton and filter paper.   When cool, each was titrated at room temperature and then again after the same sample had been boiled one minute, that is, the same procedure was followed as in titrating the meat infusions (page 214).

The following table shows the reactions of eight peptones, including Witte's.

TABLE II

Reaction of peptones (titrated with phenolphthalein)

KIND	ROOM TEMPERATURE FIGURE (AT 20 °C.)	RISE AFTER BEING BOILED ONE MINUTE IN CASSEROLE	BOILING TEMPERATURE FIGURE
Armour..................................	+0.6	+0.4	+1.0
Atkinson................................	+0.4	+0.2	+0.6
Difco...................................	+0.6	+0.2	+0.8
Eimer & Amend..........................	+1.0	+0.4	+1.4
Fairchild culture........................	+0.7	+0.4	+1.1
Leitz...................................	+0.4	+0.3	+0.7
Squibb.................................	+0.3	+0.1	+0.4
Witte..................................	+0.3	+0.1	+0.4

The peptone solutions were then divided into two sets (as with the meat infusions), one corrected and the other uncorrected. These were given successive treatments in the autoclave and titrated at the same intervals. As with the meat infusions there was a steady increase in acidity though not in so marked a degree. Three uncorrected portions of peptone solutions (Fairchild's, Eimer and Amend, Squibbs) were also run an additional six hours, making fourteen hours in all in the autoclave. As with the meat infusions the limit of hydrolysis was not reached.

In the corrected portions the total amount of acidity, developed by heating after successive additions of normal soda, again paralleled closely the rise in acidity of the corresponding uncorrected portions.

From the above it is apparent that the introduction of a peptone into a medium will affect the reaction to some extent.

INDICATORS

The shade of phenolphthalein suitable for a correct end point varies greatly in the opinion of different authors.

Miur and Ritchie (1913) give "the first trace of pink."

Hiss and Zinsser (1914)—"faint but clear and distinct pink."

Stitt (1913)—"a delicate pink (hot titration) a purplish violet color (cold titration)."

Jordan (1914)—"Faint but distinct pink color."

Park and Williams (1914)—"Faint, but distinct pink which remains on re-heating."

Heinemann (1911)—"Faint but decided and stable pink."

Abel (1912)—"Brilliant red." (Translation.)

Abbott (1915)—"Pink color."

MacNeal (1914)—"Faint but distinct and permanent pink."

Swithinbank and Newman (1903)—"Clear bright pink color."

Mallory and Wright (1915)—"Bright pink color" not "the pinkish darkening of the fluid which preceeds it."

Eyre (1915) uses a "pinkish tinge" or "a faint rose-pink which cooled to 30° or 20°C., becomes more distinct and decidedly deeper and brighter" resembling a "deep magenta color."

These shades given differ more or less from the Standard Method (1913).

That no two people seem to titrate to exactly the same shade has often been shown in our laboratory when a different worker in the media room has attempted to set the final reaction of some special medium. If the method of titrating is to be at all accurate, it is necessary to *assume* a shade of pink for phenolphthalein. This necessity is brought out by such great discrepancies between different workers titrating the same substance as are given by Clark (1915).[19]   As a help, a practical color scale may be of aid in determining the most suitable shade for a certain kind of work and approximating it as closely as possible.   This should eliminate the personal factor to some extent.

As stated before, in our opinion, the correct shade for a delicate end point in pale broth or other solutions with little color is the first most delicate pink tinge observable throughout, remaining at least one minute.

With us, when titrating agar, a mixture of 5/20 red, 3/20 orange and 12/20 white on the color-top (see page 210) has proved to be a desirable shade for the first and second titrations of neutral agar;[20] while the third titration, when the medium comes out of the autoclave, should give (on the addition of phenolphthalein) a shade consisting of 3/20 red, 3/20 orange and 14/20 white on the color-top.   These shades differ from the ones given above both in the Standard Method of titrating at boiling point (page 210) and our own definition (page 213) but they have yielded very good results.   However, it may be as difficult to decide on an end point by means of a color-top or scale as to imagine an end point from the descriptions of the various writers.

LITMUS

Since any medium with meat infusion as a basis and peptone added is a most complex mixture, no one indicator shows all

[19] (16) page 117.   Such relatively great discrepancies are surprising especially among the chemists.

[20] The deeper color of agar as compared with the usual color of broth makes necessary the use of some orange in this scale.

of its varying acid constituents. In the last 10 or 15 years phenolphthalein has been largely employed, yet it cannot be relied upon in every instance.

Park and Williams[21] state:

Different indicators differ not only in delicacy but in the substances to which they react. A medium alkaline to litmus is acid to phenolphthalein showing that there are present substances possessing a character which litmus does not detect, weak organic acids and organic compounds, theoretically amphoteric but in which an acid character predominates.

Thus a liter of bouillon becomes, on the addition of 1 per cent of peptone, more alkaline to litmus but decidedly more acid to phenolphthalein; 1000 cc. of water with 1 per cent peptone is acid to phenolphthalein to such an extent that 3.5 cc. of deci-normal NaOH is required to neutralize it. To litmus it is alkaline and requires 3.4 cc. of deci-normal HCl. Two per cent peptone doubles the difference. The same figures hold approximately true for peptone broth.

Eyre (1915) states that although meat infusion is always acid to phenolphthalein it may react neutral or even alkaline to litmus; again, if rendered exactly neutral to litmus, it still reacts acid to phenolphthalein; that this is due to the facts:

(1) Litmus is insensitive to weak organic acids whose presence is readily indicated by phenolphthalein.

(2) Dibasic sodium phosphate which is formed during process of neutralization is a salt which reacts alkaline to litmus but neutral to phenolphthalein.

On the other hand, MacNeal (1914) considers litmus the more useful:

The neutral point indicated by litmus is very nearly the actual point in respect to acidity and alkalinity, and this point is not appreciably displaced in either direction by the addition of a neutral mixture of a feebly dissociated acid and its salts to the solution. The end reaction indicated by phenolphthalein when it turns pink is actually a point at which there is a slight excess of alkali. This is so nearly the neutral point in inorganic solutions, when electrolytic dissociation

[21] Third and fourth editions—1908 and 1910.

is marked, that the error is not appreciable. In solutions of organic substances, especially when considerable amounts of feebly dissociated substances such as are contained in peptone or gelatin, are present, this error becomes very appreciable. The discrepancy between the end point for litmus and for phenolphthalein will vary for different lots of media.

Naturally those media which contain litmus as an indicator to show acid production by the growth of bacteria, must be alkaline to litmus yet not too alkaline or the indicator is rendered useless. The testing of such media by the use of litmus paper is an unsatisfactory and crude method useful for only the roughest work. The use of a litmus solution (Merck's purified in 5 per cent aqueous solution) is far more satisfactory. "Neutral to litmus" is "so and so" acid to phenolphthalein, the figure given varying with the writer. Muir and Ritchie place it at + 2.5, Stitt at + 1.5 boiling titration and about + 0.7 with the cold titration. Abbott gives + 2.5, Abel + 1.5 to 2.5 and Heinemann + 2, all depending on the shade of pink considered by the worker as suitable and the length of time the sample is boiled in the casserole.

In the modified method used in our laboratory, the figure is as low as + 0.6 or + 0.7 with media prepared with 1 per cent peptone. (This figure rises to + 1 when the boiling titration is used.) A 1 per cent peptone (Witte) solution in water is about + 0.2 to + 0.3 with phenolphthalein at room temperature and about + 0.4 at boiling figure.

It has been shown by Hildebrand (1913) and others (Clark, 1915; Bovie, 1915) that an indicator does not indicate the point of actual neutrality but merely a definite degree of hydrogen ion concentration. Where these two points coincide, i.e., where the hydrogen ion concentration at which the indicator changes color, is within the zone of absolute neutrality for a particular mixture of substances, this change of color in an indicator will, of course, be of significance. It would therefore seem desirable to select a specific indicator for each class of media. This could be accomplished only by determining the effect of added

alkali upon the electrical conductivity of the particular medium. Such measurement should be made by means of the hydrogen electrode. The measurements of potential are usually expressed in terms of hydrogen ion concentration, that is, acidity.

For an apparatus as described by Bovie (1915) the details of manipulation are as follows: A mixture of the medium with water in the same proportions as used for ordinary titrations is placed in a beaker kept at 30°C., and the precaution of excluding CO_2 observed. The indicator is added and the standard electrodes are immersed. Successive portions of decinormal sodium hydroxid solution are then added. After each addition of the alkali the potential of the mixture is observed and recorded.

The point at which the indicator gives its first change of color is marked. The additions of alkali should be continued and the potentials further noted until the curve changes its shape—that is, from concave to convex or vice versa. The actual point at which this change takes place is known as the point of inflection and is a true neutral point. The nearness of the indicator's point of change to this point of inflection determines its suitability for this particular class of media. The indicator in which the change comes nearest to this point should be selected for practical use. Work in choosing such indicators according to this method is planned.

When titrations are performed under ordinary circumstances at the boiling point, it is noticeable that making a decision as to the correct end point to phenolphthalein is much more difficult than when the temperature of 20° to 30° C. is used. This is due to the changes of ionization in the mixture caused by this considerable change in temperature.

Besides this, the constant presence of colloidal substances[22] in peptones,[23] phosphates and sugars in all media gives rise to

[22] Hildebrand (1913), Clark (1915) and others have shown that colloidal substances affect the sharpness of indicator end points.

[23] In the titration of the peptone solutions it was very difficult to get a sharp end point even at 20°C. The indicator (phenolphthalein) seemed to "flare" suddenly from the colorless state to a deeper pink than the one showing a really delicate end point.

a further depression of ionization, especially when these substances are decomposed by boiling.

The meagre results from the few investigations conducted in this field lead us to suspect that the presence of sugars in media may have a considerable influence on the effective acidity, that is, the dissociated acid principles (Hildebrand 1913). Work in these lines is to be continued.

SUMMARY

Marked and continued hydrolysis, resulting in the formation of acid principles, occurred on successive heatings of meat infusions in the autoclave. The state of complete hydrolysis, i.e., the point at which no further acidity is produced, was not reached with these meat infusions (which had been subjected previously to boiling over the open flame for one to two hours) even after prolonged autoclaving at fifteen pounds pressure for eight[24] hours.

Hydrolysis did not occur in solutions of Liebig's beef extract subjected to similar heatings. In the portion which had been adjusted to the neutral point, however, slight hydrolysis did take place.

Those portions of meat infusion in which the natural acidity had been adjusted with normal sodium hydroxid to plus one (phenolphthalein) showed that hydrolysis occurred on application of heat and continued to do so after successive adjustments and autoclavings. There was produced in these portions as much acidity, approximately, as was developed on heating the corresponding uncorrected portions.

The further production of acidity after the addition of sodium hydroxid is due to the hydrolytic effect of heat in the presence of water, upon these portions of the medium unbound by alkali.

The fact that hydrolysis is promoted by heat makes inaccurate the estimation of acid ions in a batch of medium when there is

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[24] With three of the meat infusions the time was extended from eight to fourteen hours.

taken as an index a sample[25] titrated at the boiling point with phenolphthalein as the indicator.   Boiling has also a marked effect on the ionic concentration in media mixtures, hence the boiling titration called for in the Standard Method and followed more or less closely by many workers and recommended in the various text books, is subject to greater error than titrations performed at a temperature of 20° to 30° C.

In the adjustment of culture media not only is the desired end point more closely approximated by titration at 20° to 30° C. (see chart 6), but the misleading information of the boiling titration is avoided (see chart 4).   Although according to Eyre (see page 211) certain acids are detected only at the boiling point, the *actual* reaction of media at the temperatures at which they are *used* is the object of vital importance.   The conditions prevailing at these temperatures (incubator, 37.5°C., for some organisms and room temperature for others) are more closely indicated by titrations conducted at room temperature.

As shown in chart 4 the actual reaction of a medium titrated by the boiling method is really from 0.5 to 1 per cent lower than is indicated by the boiling titration figures.

The tests made on the reaction towards phenolphthalein of the various peptones on the market showed them to differ greatly in acidity.   The effect on peptones of prolonged heating in tests similar to those on the meat infusions showed that hydrolysis occurred on the application of heat.   The development of acidity took place after successive adjustments of reaction with sodium hydroxid, as with the meat infusions and the total amount of hydrolysis approximated closely that of the uncorrected portions.   Here, too, prolonged heating of fourteen hours in the autoclave did not give complete hydrolysis.

In the usual titration methods, no one indicator gives all the

[25] That is, if the titration is performed after a preliminary boiling of the whole batch of meat infusion.   When a titration is made on meat juice pressed out in the cold and containing added peptone dissolved at a low temperature, the boiling of the sample in the casserole is necessary to approximate future conditions after the boiling of the whole batch.

evidence desired in every case, e.g., the differences between litmus and phenolphthalein.

The necessary dependence on the change in color of an indicator to show the reaction of a solution, especially in the case of culture media, gives rise to error. This is due partly to the depressing effect of colloids, phosphates and some sugars which affect the sensitiveness of indicators; and also to variations of judgment with different workers as to the correct shade of color for an end point. Where it is desirable to avoid these influences titrations may be made by measurement of the electrical potential.

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A NEW SPECIES OF ALCOHOL FORMING BACTERIUM ISOLATED FROM THE INTERIOR OF STALKS OF SUGAR CANE INFESTED WITH THE CANE-BORER *DIATRAEA SACCHARALIS*

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Among the injuries sustained by sugar cane from various insects, that resulting from infestation with the sugar cane moth borer, *Diatraea saccharalis*, is generally regarded in Louisiana as of greatest importance. This pest is very widely distributed throughout the sugar cane growing countries of the world. Holloway (1912) who has made a very extended study of the occurrence of this insect in Louisiana, reported an average infestation of the crop of 1911 to be about 38 per cent. This infestation varied in degree in different sections, ranging from 0– to 78 per cent. The nature of the injury wrought upon sugar cane by this parasite is manifold. Barber (1911) attributed a greatly impaired germinating power of the cane to the injury of its eyes from the burrows made by the borer. Borer infested cane is also stunted in growth, and is rendered less able to withstand high winds, while its value is further impaired by the secondary infection of its interior by various fungi.

Of these various injuries caused by borer infestations, the last named is perhaps the most serious. Barber (1911) found the decrease in the purity of such cane to amount to 5.6 per cent. On the basis of recoverable sucrose per acre of cane, the above deterioration would amount to over 1000 pounds of sucrose for every 25 tons of cane.

Van Dine (1912) in investigating the borer injury to sugar cane in Porto Rico, reports a decrease of 5.8 in the purity of the juice, resulting from infestation with this parasite.

Among the secondary injuries to borer infested cane induced
by fungi, those resulting from the infection with the Red Rot
disease, is of first importance in this State.   Of this injury, as well
as of the general nature of the disease and the fungus, Edgerton
(1911) has made a very extended study.   That this fungus
plays a most important part in the deterioration of the juice
of canes infested with the borer, may be judged from the fol-
lowing analyses in Table III of the publicationof the above author.

CONDITION OF CANE	SUCROSE	GLUCOSE
	per cent	*per cent*
Sound...	10.50	2.30
Borer cane....................................	11.40	1.90
Borer cane with red rot.......................	7.80	3.03

As the greatest infestation of cane with the red rot disease
occurs as a sequel to borer attacks the economic importance of
the control of this insect is obviously very great.

It would appear that the burrows made by the cane borer
might offer suitable surroundings for the development of many
species of bacteria which would inevitably find their way into
the interior of the cane stalks.   With a view of determining the
part played by bacteria in causing a deterioration of the juice
of borer infested cane, the writer began a preliminary investi-
gation of this subject in the fall of 1914.   At that time a large
number of borer infested canes were examined, and an attempt
made to isolate the species of bacteria occurring therein.   The
canes were brought to the laboratory, cut into short sections,
and the portion of the stalk surrounding the borer wounds,
washed off in a 1:1000 bichloride of mercury solution.   A sterile
platinum loop was then inserted as far into the interior of the
wound as possible, and the injured tissue transferred to tubes
of sterile culture media.   As it happened the media employed
for this purpose contained 10 per cent of sucrose.   After a short
incubation period, an examination of these tubes showed that
practically every one of them was undergoing a vigorous fer-
mentation.   The presence of yeast cells in the tubes was quite

naturally expected, but a microscopical examination showed that bacteria rather than yeasts were predominant. Transfers to sterile plates, and the isolation of the predominating bacterial species, resulted in obtaining a culture, which showed a marked ability to ferment sucrose solutions. The copious amount of gas given off, which was tested for CO_2 with positive results, and the odor of alcohol, suggested the capacity of the species to induce an alcoholic fermentation. When transferred to sterile glycerine bouillon, the species also induced a vigorous fermentation, indicating its possible relationship or identity with other species of bacteria described in the literature. Although several species of alcohol forming bacteria have been isolated, this property may be regarded as rare. Among the most prominent of these species the following may be mentioned. *B. Fitzianus* was isolated from a cold hay infusion by Fitz (1880) and later more closely studied, and named by Buchner. This species forms ethyl alcohol from glycerine. Frankland and Fox (1889) isolated from the solid excreta of sheep an alcohol forming species of bacteria to which they gave the name *B. ethaceticus*. This species forms ethyl alcohol and acetic acid from glycerine. According to the work of Friedlander (1911) *B. pneumoniae* has the power of forming ethyl alcohol and acetic acid in nutrient solutions containing sucrose. Kruis and Rayman (1895) isolated from sour yeast a lactic acid bacterium that forms ethyl alcohol as a by-product.

Other species of alcohol forming bacteria are Duclaux's (1895) *Amylobacter ethylicus* isolated from garden soil, *B. butylicus* isolated by Fitz (1884) and two species isolated from malt decoctions by Henneberg (1909). There seems to be much in common between the characteristics of these species and our Louisiana organism, yet there are sufficient differences clearly to differentiate the latter from the former.

The *characteristics* of the sugar cane bacterium are as follows:

Morphological. Short thick rods with rounded ends, the individual cells averaging 2.8 μ in length and 1.0 μ in breadth. The rods occur chiefly in pairs, are frequently single, never in chains. The cells stain readily by aqueous and alcoholic solu-

tions of aniline dyes, and are Gram positive. The rods are non-motile, non-flagellated, and do not form endospores.

Physiological characteristics. This species does not liquefy gelatine at all. Milk is rendered slightly acid, and gas is developed after an incubation of 24 hours at 35°C. The consistency of the milk is unchanged in three days. Nitrates are not reduced. A fairly good growth occurred in a Novy jar from which all of the air was exhausted by means of a vacuum pump, and with the bottom covered with pyrogallic acid solution. The species is therefore a facultative anaerobe.

The following sugars are fermented by this species:

	SUC-ROSE	GLU-COSE	LEVU-LOSE	MAN-NITE	LAC-TOSE	GALAC-TOSE	RAFFI-NOSE	MALT-OSE	GLYC-ERIN
Gas...............	+	+	+	+	+	+	+	+	+
Growth in closed arm.............	+	+	+	+	+	+	+	+	+

Cultural characteristics. On plain agar the colonies are small, rounded, but slightly raised, with entire edges. The surface is smooth and moist, with an amorphous interior structure. On glycerin agar the colonies are round greyish white, with a more glistening surface, but otherwise similar to the colonies on the plain agar. The sub-surface colonies are surrounded by gas bubbles resulting from the fermentation of the glycerin.

On agar streaks the growth is exceedingly rapid. Inoculated tubes show a marked growth along the line of the needle after six hours' incubation at 35°C.

On potato the organism forms a dirty white echinulate growth, slightly raised, with a glistening lustre. The growth is of a butyrous consistency. In bouillon the growth is very rapid, and the solution quickly becomes cloudy throughout. No film is produced. In bouillon containing 2 per cent of sucrose a vigorous fermentation follows inoculation with this species. The medium is rendered acid, and the acidity on the third day gives an acidifying coefficient of 3.8.

In its morphological and physiological characteristics the species in question strikingly resembles other species previously

isolated. In the table given below and in that on page 240 will be found the most prominent distinguishing features.

CHARACTERS	SUGAR CANE ORGANISM B. SACCHARALIS	BAC. FITZIANUS (FITZ)	BAC. ETHACETICUS (FRANKLAND)	BAC. ETHACETO SUCCINICUS	HENNEBERG BACILLI	
					1	2
Size....... {	2.8 μ long 1.0 μ broad	Very large; similar to B. subtilis	1.5–5.1 μ L 0.8–1.0 μ B	1.7–1.5 μ L 0.5–1.0 μ B	—	Small short rods
Spore formation....	—	+	—	—	—	—
Flagella.....	—	+ (Probable)	+ (Motile)	+ (Probable)		+ (Motile) (+)

From the foregoing table of characteristics of the various species of alcohol forming bacteria, and from that on page 240, it will be noted that the species isolated from sugar cane, differs from the others in the following essential points.

FROM BAC. FITZIANUS	FROM BAC. ETHACETICUS	FROM BAC. ETHACETO SUCCINICUS	FROM HENNEBERG BACILLI	
			1	2
In smaller size and absence of spores	Not liquefying gelatine; absence of motility	Growth on agar and gelatine; absence of motility	Non-liquefaction of gelatine; absence of motility	Absence of motility; strong fermentation of sucrose, glucose, and levulose

The observed differences in the characteristics of this species, seem sufficient to constitute it as a new species. Owing to its prevalence in borer infested sugar cane, the name *Bacillus saccharalis* seems appropriate.

In order to determine the amount of alcohol formed by this species in the fermentation of glycerin, a solution was prepared according to the formula of Frankland and Fox (1889), which is as follows:

<div align="right">grams</div>

Glycerin.. 60
Peptone.. 2
Calcium carbonate (precipitated)................................ 30

Cultural characters

CHARACTERS	BAC. SACCHARALIS	BACILLUS FITZIANUS	BACILLUS ETHACETICUS (FRANKLAND)	BACILLUS ETHACETO SUCCIN.CUS	HENNEBERG BACILLI 1	HENNEBERG BACILLI 2
Colonies on plain agar	Round white raised, glistening	—	Thin veil-like growth almost invisible	—	—	—
Colonies on gelatine	Small round white; no liquefaction	Brownish yellow. No liquefaction	Small white dots. Medium liquefied	Yellow. No liquefaction. Colonies thin spreading	—	—
Agar streak	White glistening growth. Confined to needle track	—		—		
Agar stab	Non-characteristic. Surface growth white. Line at puncture echinulate*	—		Thick growth white surface	—	
Gelatine stab	Line of stab filiform. No liquefaction	—	Beaded appearance; rapid liquefaction	Thin non-characteristic growth	Gelatine liquefied	
Potato	Vigorous raised moist white growth	—	Dirty white shining growth covering entire surface	Thick vigorous spreading growth yellow		
Bouillon	Cloudy white sediment deposited on bottom of tube	—	White pellicle; white sediment		—	—

* Echinulate.

Dissolved in 2000 cc. of the following salt solution:

	DISSOLVED IN 5000 CC. WATER grams
Potassium phosphate	5.
Magnesium sulphate	1.0
Calcium chlorid	0.5

The solution was divided into one liter portions sterilized by the intermittent method, and inoculated with a pure culture of *Bacillus saccharalis*.   After an incubation period of two weeks, an alcohol determination was made.   The liquor was evaporated down to about a third of the original volume, until the distillate gave only a faint reaction with iodoform.   After repeated distillations, the specific gravity of the 50 cc. portion was found to be 0.99744 which corresponds to 1.707 per cent of alcohol by volume.   By dehydrating a small portion with fused carbonate of potash a solution was obtained which distilled at 79° to 80°C. showing it to be ethyl alcohol.

The residue was tested for acids and acetic acid was found to be present, using the ethyl acetate test.   A mannite solution made up according to the same formula as the glycerin solution, except that 3 per cent of mannite was substituted for an equal weight of glycerine, was next tried.   It yielded 50 cc. of a distillate with a specific gravity of 0.99836 corresponding to an alcohol per cent of 0.55 by volume.   The presence of acetic acid was also detected in the residue.

Thinking that the low yields of alcohol in the two cases was due to the small quantity of assimilable nitrogen in the solution, 3 per cent of glycerin was added to plain bouillon, and the flask sterilized and inoculated as before.   In this case the solution yielded 50 cc. of distillate of a specific gravity of 0.9864, which corresponds to an alcohol per cent of 4.895, which was much higher than in the previous experiment.   The higher yield in the latter case indicated that there was a lack of nitrogen in the solution previously used.   Frankland and Fox in their experiment with *B. ethaceticus* obtained a yield of 11.41 grams of alcohol from 60 grams of glycerin.   In the experiments of the above investigators it was found that *B. ethaceticus* formed

1.63 parts of alcohol to one part of acetic acid, from mannite, while from glycerin the ratio of alcohol and acid was 2.11 to 1. Although *B. saccharalis* also forms alcohol and acetic acid from mannite, the ratio in which these products are formed was not determined in our experiments.

ACTION ON SUGAR CANE JUICE

B. saccharalis grows vigorously in cane juice, and apparently induces a strong fermentation of its sugars. In order to determine its effect upon the composition of this substance, sterilized cane juice was inoculated and the following results were obtained. To one flask 1 per cent $CaCO_3$ was added, in order to neutralize acids formed during fermentation.

SAMPLE	TREATMENT	TOTAL SOLIDS	INVERT SUGAR	SUCROSE		ACIDITY	ALCOHOL PER CENT VOLUME	PURITY
				Single polarization	Clerget			
				per cent	*per cent*			
I	Inoculated + 1 per cent $CaCO_3$..........	7.75	0.73	4.4	4.86	1.2	0.53	56
II	Control + 1 per cent $CaCo_3$..............	12.73	3.7	5.5	6.91	1.2		43
III	Inoculated.............	8.75	1.3	4.2	5.92	2.8	0.79	47
IV	Control................	12.43	4.27	5.7	7.36	1.8		45

It will be noted from the above table that while some of the sucrose of the juice is inverted by the organism, a larger quanity of reducing sugars is destroyed. This results in an apparent increase in the purity of the inoculated flasks over the controls.

The question of what rôle this species plays in growing cane, and what effect its presence exercises on the composition of the juice of such cane, led to some inoculation experiments being conducted in 1914 and 1915 in the fields of the Sugar Experiment Station. In the first series of experiments the cane was inoculated by means of a small cork borer, and a pipette. The inoculations were made in the following manner. Holes were made in the cane with the cork borer, and 5 cc. of a water sus-

pension of a 24 hour-agar streak of the organism, was then introduced. The controls were treated in a similar manner, except that 5 cc. of sterile water was used instead of a culture. The holes in the cane were then sealed with grafting wax. All of the canes slected for the experiment were first examined for borer infestation, and only the borer free canes were used. About twenty inoculations were made in the first experiment, the varieties D. 74 and D. 95 being selected for the purpose. The inoculations were made on the 12th of October, and the canes were analyzed about the first of December, allowing nearly two months for the bacteria to develop. When the canes were analyzed, they were split through lengthwise, and transfers made with a sterile platinum loop, from the inoculation wounds to sterile glycerine bouillon. In the majority of cases the *B. saccharalis* was recovered from the inoculated portion of the cane, showing that it had remained in a living condition within the cane. The analyses of the canes were so variable, that it was decided to repeat the experiment the following year, using a slightly different method of inoculation. In September of the following year two rows of D. 74 cane were inoculated. Instead of a water suspension of the organism, a three days' old culture grown on sterile mashed potato was used as the inoculating material, and a blackleg vaccine injector was employed for the inoculations. The analysis of the cane was made in November, thus allowing an incubation period of two months for the organism to carry on its activities within the cane. The results of the analyses again showed that there was no marked deterioration of the juice of the inoculated cane. The juice of the inoculated cane, it is true, showed an average purity of 64.3 as against 67.4 for the control, but there were as many cases where the purity of the inoculated cane was higher than its control, as where it was lower. In this experiment, just as in the previous one, the organism was recovered from the inoculated portion of the cane, showing that it had remained in a living condition during the entire period. It is possible that the variations in the composition of the juice from different canes, even though they may be of the same size and in the same

stool, may have accounted for the negative results of the inoculation experiments. It is likely, however, that *B. saccharalis* does not induce any marked deterioration of the juice of growing cane, and indeed the apparently negative results which indicated a higher purity in the inoculated canes is well within the range of possible results from the action of the species. We have seen in the experiment on the action of this species upon cane juice how an increase of the purity of the juice may result from the fermentation of the invert sugar. It seems very probable that a similar result might follow from the presence of the species in growing cane. The occurrence of *B. saccharalis* in borer infested cane, and its survival in the interior of cane artificially inoculated with it, suggests a certain ability on its part to protect itself against the defensive properties of the plant. Sugar cane, like all other plants, possesses protective enzymes which tend to prevent the invasion of its tissues with organisms and their development therein, once they succeed in gaining an entrance. Browne (1906) reports a distinctly germicidal property of freshly extracted cane juice. He says:

The darkening of vegetable tissues on the exposure to the air has been explained by Bertrand, to be due to the action of an oxidizing enzyme upon various tannin bodies, all more or less related to the polyphenols, and the query naturally arises does cane juice itself exercise any germicidal properties in connection with the natural phenomenon of darkening. The conclusion which we have reached in investigating this point is that cane juice does acquire for a time such germicidal characteristics. Counting the bacteria in the expressed juice of the cane at regular periods usually shows for several hours a uniform decrease in numbers; with juice from sterilized canes on the other hand, the bacterial content increases from the very start.

Again the author referred to states, that

The living plant therefore does appear to protect itself against the invasion of microscopic parasites by forming toxic products.

The relation between the germicidal power of cane juice and the enzymes it contains, is suggested in the following observation by Browne, viz.,

The test for oxydase and catalase in cane juice becomes very feeble after ten or twelve hours, and with the disappearance of enzymic power, the number of bacteria begins to undergo a sudden increase. But it is more especially within the body of the cane itself that this germicidal action is most evident, and this we might expect not only from the colloidal and adherent character of the enzymes which renders them resistant to expression, but from the facts of localization, etc.

From this we must conclude that *B. saccharalis* possesses certain defensive properties which enable it to develop in spite of exposure to the germicidal action of the enzymes within the interior of the sugar cane. The prevalence of this interesting species in the interior of · borer infested cane, and its predominance therein, further emphasizes the ability of *B. saccharalis* to overcome the defensive properties of the plant.

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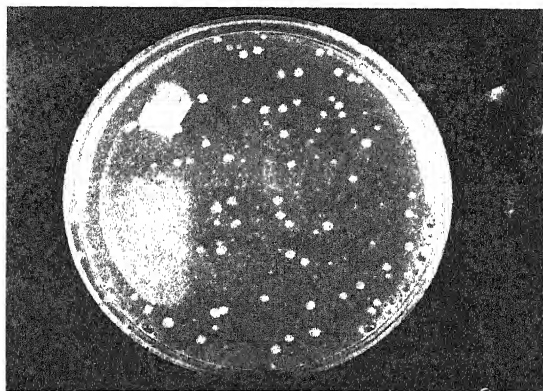
Fig. 2. A twenty-four hour growth of *B. saccharalis* on plain agar.
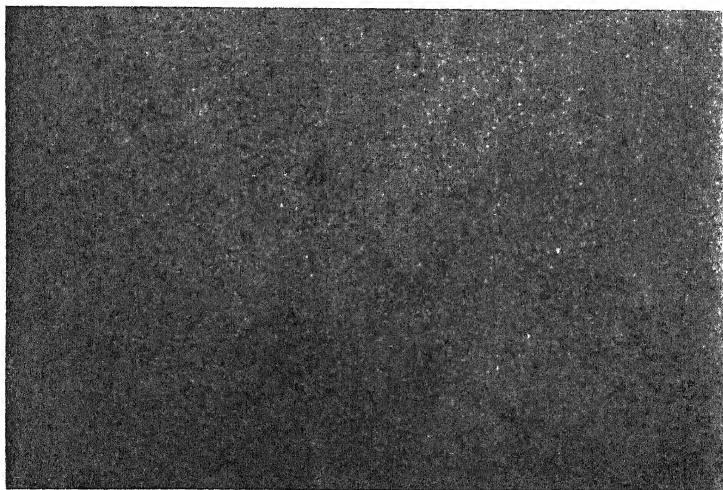
FIG. 1. A photomicrograph from a twenty-four hour agar growth of *B. saccharalis*.
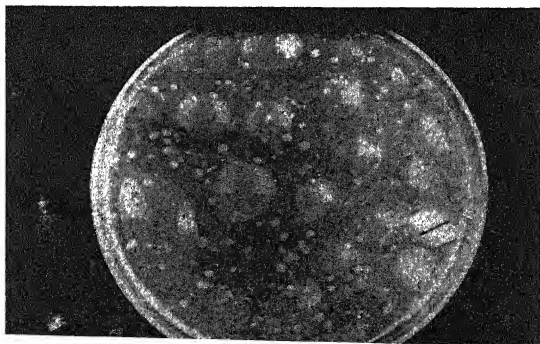
ABSTRACTS OF AMERICAN BACTERIOLOGICAL LITERATURE

ANIMAL PATHOLOGY

The Maintenance of Virulence of Bacillus abortivus equinus. E. S. GOOD AND W. V. Smith. Jour. Med. Res., 1916, *33*, 493–498.

The authors present a note on the ability of the above bacillus to retain its virulence when kept under artificial cultivation over a long period. Inoculation of 1 cc. of a mixture of eight strains, representing only one-fifth of an agar slant, produced typical abortion in a mare protected with 200 cc. of hyperimmune serum. The strains used in this experiment had been isolated for periods varying from three to five years.—H. W. L.

Studies to Diagnose a Fatal Disease of Cattle in the Mountainous Regions of California. K. F. MEYER. Jour. Am. Vet. Med. Assoc., 1916, *48*, 552–560.

Discussion of the subject is divided into symptomatology, anatomical findings, bacteriological examinations and epidemiology.

Pieces of organs forwarded to the laboratory were subjected to microscopic examination without revealing bipolar organisms that could be regarded as *Bacterium bovisepticum* nor did ordinary culture methods give satisfactory results. Of about twenty-five rabbits inoculated with emulsions of liver infarcts, lymph nodes and spleen material, two died from a typical bipolar infection. The cultures isolated from these rabbits gave all the cultural identity reactions recognized as typical for *Bacterium bovisepticum.* The pathogenicity tests were characteristic except that large doses were necessary to produce fatal results. A three weeks old calf succumbed 22 hours after the intravenous injection of 3 cc. of a 20 hour old broth culture. Both strains were identical and serologically protected against each other and against strains of *Bacterium bovisepticum* from various sources in the United States.

Inoculation of guinea pigs with liver and infarct material caused death from infections with an undetermined anaerobe, *Bacillus coli* and diplococci. The anaerobe was not pathogenic to calves. Most of the mice inoculated with similar material remained alive or succumbed to the same anaerobe as did the guinea pigs.

The writer does not feel that the evidence thus far collected is sufficient to make a conclusive diagnosis of hemorrhagic septicemia but as a working hypothesis has assumed that the disease in all probability is hemorrhagic septicemia. The difficulties experienced brought forward again the fact that the bacteriological diagnosis of hemorrhagic septicemia is not as easy a procedure as is generally considered.—A. R. W.

Vaccination Experiments Against Anthrax. A. EICHHORN. Jour. Am. Vet. Med. Assoc., 1916, *48*, 669–686.

The writer reviews the development of measures for protecting animals from anthrax by such means as Pasteur's vaccination, spore vaccines, and injection of a serum as prepared by Sobernheim. The latter showed that the injection of an immune animal with increasing amounts of virulent virus would produce a serum possessing great protective value against anthrax. The author draws the following conclusions from his work with spore vaccines and serum:

1. Horses are suitable for the production of highly potent anthrax serum. Serum of such horses should protect large animals in 10 cc. doses.

2. The use of the serum treatment alone is indicated in cases where the infection has already occurred in a herd. Since the serum confers only a passive immunity it is advisable to revaccinate the herd in from three to five weeks by the simultaneous method.

3. The serum possesses great curative value. Depending on the severity of the infection, the curative dose is from 30 to 100 cc.; the injection may be repeated if necessary.

4. For the simultaneous treatment, a spore vaccine carefully standardized, is preferable to the ordinary Pasteur vaccine.

5. Spore vaccine should be employed in preference to the Pasteur vaccines for immunization with vaccine alone. The possibility of more accurate dosing of the spore vaccine and the better keeping qualities of the same, give this product a decided advantage over the other.

6. Experiments with concentrated serum and dry spore vaccine are very promising. This method would greatly simplify the vaccination process and also insure the product against subsequent contamination and deterioration.—A. R. W.

BACTERIOLOGY OF WATER AND SEWAGE

The Fundamental Principles of the Activated Sludge Process of Sewage Treatment. T. CHALKLEY HALTON. Indiana San. and W. S. Assn., 1916, Eng. Contrg. *45*, 235–236.

The activated sludge process depends on the presence of biological life in the sludge under aerobic conditions.—L. P.

Sanitary Features of Los Angeles Aqueduct. B. A. HEINLEY. Mun. J., 1916, *40*, 35–37.

The water is brought 233 miles from Owens River to Los Angeles. The density of the population in the Watershed is 1.4 per square mile. In addition to the time in the aqueduct, reservoirs increase the normal storage period of 65 days under present conditions to 468 days. *B. coli* noted were traced to ducks. The mineral content ranged from 15 to 22 grains per gallon. Algae growths occasionally cause odors and taste, despite covered reservoirs on distribution system.—L. P.

The Activated Sludge Process of Sewage Treatment. G. J. Fowler. Can. Eng. 1916, *30*, 227–228.

The author sketches the historical development of the process, and dwells on the "M7" process of adding bacterial cultures in the presence of iron in solution. Activated Sludge has 3 general effects (1) a clotting or clarifying action (2) a rapid oxidation of carbon and (3) nitrification. Much research is still required.—L. P.

Hartford (Conn.) Waterworks Notes. C. M. Saville. Report Bd. Water Commrs., 1915, Mun. J., 1916, *40*, 333–334.

On account of the proximity of highways to reservoirs the water is sterilized before delivery. The raw water shows bacterial counts as high as 39,000 per cubic centimeter with *B. coli* found from 9 to 23 times in 10 cc. or less, every month. Treatment with 0.95 part per million available Cl has removed *B. coli* and 99.8 per cent of total bacteria. With CaOCl₂, 1 part per million available Cl was used or 25 pounds of bleach per million gallons. Liquid Cl used, 0.65 p.p.m. or 5.4 pounds per million gallons.—L. P.

Vitality of the Cholera Vibrio in the Water of New York Bay. A. J. Gelarie. Medical Record, 1916, *89*, 236.

The question whether the cholera vibrio dies or survives in native bay water is of importance in view of the fact that the waters about the Quarantine Station in New York may at any time be open to infection. Accordingly, a series of experiments was carried out to determine the viability of the cholera vibrio in native bay water.

Preliminary work demonstrated that the subjection of cholera vibrios to the osmotic pressure of bay water had no apparent effect.

Other organisms present in bay water were found to have an inhibitory influence upon the growth of the cholera vibrios. Vibrios not previously enriched with peptone were eliminated after 48 hours, those receiving preliminary enrichment after 7 to 47 days, the period varying according to the strain of cholera employed, the character of the water, and the quantity of bacteria added. Cholera vibrios added to sterilized bay water were found alive in some cases at the end of 285 days.

The demonstration of live vibrios in native bay water after a period of 7 to 47 days proves conclusively that every precaution should be taken to prevent pollution of bay waters.—M. W. C.

IMMUNOLOGY

Tuberculin Therapy. Henry L. Shively. New York Med. Jour., 1916, *103*, 51.

General discussion of tuberculin therapy with report of three cases.—M. W. C.

Experimental Study of the Effect of Emetinized Blood on the Typhoid Bacillus. MARCUS BEEKMAN. Medical Record, 1916, *89*, 284.

The subcutaneous administration of emetine hydrochloride in 0.5 grain doses every six hours does not impart to the blood any bactericidal properties for the typhoid bacillus.—M. W. C.

Newer Practical Points in the Treatment of Typhoid Fever. BEVERLEY ROBINSON. Medical Record, 1916, *89*, 311.

In a discussion of the newer methods of treatment of typhoid fever, the advantages of vaccine treatment are considered as still questionable. —M. W. C.

Note on a Skin Reaction in Pneumonia. RICHARD WEIL. Jour. Exp. Med., 1915, *23*, 10–14.

The intradermic injection of pneumococcus autolysate in patients suffering from pneumonia produced such variations in the skin reactions that the author concludes that from a diagnostic standpoint, at least, the test has no significance.—B. W.

The Preparation and Preservation of Complement. LOYD THOMPSON. Jour. A. M. A., 1916, *66*, 652.

Fresh guinea pig complement is diluted 1:1 with an 8.1 per cent sodium chlorid solution. It is sealed in small tubes, 2 cc. to the tube. Before use 8 cc. of water is added to a tube, giving an isotonic 1:10 dilution of guinea pig serum. Complement held under these conditions is active for two weeks or longer.—G. H. S.

Frontal and Maxillary Sinusitis and Sequelae. Due to Staphylococcus pyogenes albus. RALPH OPDYKE. Medical Record, 1916, *89*, 18.

An account of a case in which a prolonged series of severe and obstinate involvements was found to be due solely to *Staphylococcus albus.* An autogenous vaccine, prepared and administered immediately after the beginning of the disease, was used without beneficial results.—M. W. C.

Recent Developments in the Treatment of Leprosy. VICTOR G. HEISER. New York Med. Jour., 1916, *103*, 289.

In reviewing the methods which have been used in the treatment of leprosy, the author states that vaccine treatment has apparently caused improvement in some cases, but in his own experience it has proved unreliable. In his opinion, the most satisfactory treatment consists of the subcutaneous administration of a mixture of chalumoogra oil, camphorated oil, and resorcin. The use of this mixture has caused cures in some cases, marked improvement in many. Examination of material taken from cured cases did not reveal the presence of leprosy bacilli.—M. W. C.

Vaccine Therapy. G. A. EHRET. Medical Record, 1916, *89*, 328.

Bacterins were used in a variety of infections—colon cystitis, bronchopneumonia, lobar pneumonia, chronic gonorrheal cystitis and prostatitis, gonorrheal arthritis, chronic articular rheumatism, neuritis, bronchial asthma, and otitis media—with successful results in every instance except one case of bronchial asthma. In the majority of cases, stock vaccines were used. The number of administrations and duration of treatment varied with the character of the case.—M. W. C.

Immunological Studies in Pneumonia. RICHARD WEIL and JOHN C. TORREY. Jour. Exp. Med., 1916, *23*, 1–10.

The authors injected guinea pigs subcutaneously with 4 cc. of the inactivated serum of pneumonia patients and from two to six days later tested the animals for hypersensitiveness by applying a pneumococcus autolysate to the excised uterus according to the method of Dale. The tests were controlled with serum from normal individuals or from patients suffering from diseases other than pneumonia. Of twenty cases of pneumococcus infection only two failed to produce sensitization, while in none of the control cases was a positive reaction obtained. The sensitizing antibody is present in the blood early in the disease and is found rarely after crisis. From the results it would appear that while the reaction is specific for the genus it cannot be used for group differentiation.—B. W.

Progress in the Treatment of Skin Diseases. G. M. MACKEE. New York Med. Jour., 1916, *103*, 441–444.

An interesting summary is given of the progress made during the last few years in the treatment of skin diseases.

Most important advances have been made in work upon the etiology of many of the dermatoses, particularly eczema.

The results reported with vaccine treatment are not uniform. Vaccine therapy has met with success in ringworm of the scalp, and in some cases, though not generally, in acne vulgaris. Bazin's disease, known as erythema induratum, and lupus vulgaris have been aided by tuberculin therapy.

Tuberculin is of no service, however, in the tuberculides nor in lupus erythematosus.—M. W. C.

The Treatment of Typhoid Fever with Bacterins. EDWARD WAITZFELDER. New York Med. Jour., 1916, *103*, 407.

Of sixteen cases of typhoid fever fourteen were treated with bacterins while two were used as controls and treated symptomatically. The bacterins used were prepared by the New York City board of health and were given intramuscularly in doses varying from 66 to 100 millions. It was found that the larger doses were the more effective.

The treatment was successful in that in the bacterin treated cases there was less fever, cardiac weakness, delirium, and exhaustion than

in the control cases. The period of convalescence as well as the period of acute illness was shortened, and in no cases were there any untoward results.—M. W. C.

Treatment of Rheumatic Fever. BEVERLEY ROBINSON. Medical Record, 1916, *89*, 11.

In discussing methods of treatment of rheumatic fever, the author mentions the use of vaccines and serums. Serums have proved to be without success and the advantages of vaccine treatment are still questionable. Whenever tried, vaccines should be used with great caution. Polyvalent vaccines should not be administered, as there is too great a risk of overburdening the system with non-specific antibodies. A further difficulty in the way of vaccine treatment is the fact that in the acute stage of the disease, the only time when bacteria can be isolated from the joints, vaccines do the least amount of good and their use is accompanied by greater local and general reactions than at a later period.—M. W. C.

Antiblastic Immunity. A. R. DOCHEZ and O. T. AVERY. Jour. Exp. Med., 1916, *23*, 61–68.

Ehrlich's side-chain theory, comprehensive as it is, fails to account for certain phenomena observed in immunological studies. Its author postulated a "third factor" to cover this decrepancy. Dochez and Avery now find that antipneumococcus serum possesses the power not only of inhibiting for a certain period the multiplication of pneumococci but also of inhibiting in varying degree their proteolytic and glycolytic functions. This power is present to a limited extent in the sera of certain normal animals, and, in human serum during the course of lobar pneumonia it appears or increases markedly at the critical period of the disease. The hypothesis that this retardation of bacterial growth is dependent upon the inhibition of metabolic function due to the presence of anti-enzymotic substances in antipneumococcus serum offers a possible explanation of the so-called "third factor" as well as a promising suggestion for further investigation.—B. W.

The Complement Fixation Reactions of the Bordet-Gengou Bacillus. M. P. OLMSTEAD and O. R. POVITZKY. Jour. Med. Res., 1916, *33*, 379–392.

Testing fourteen typical and four atypical strains of *Bacillus pertussis*, and nine strains of strictly hemoglobinophilic bacilli, by means of complement fixation, the authors report further confirmatory evidence of the individuality of *B. pertussis*, particularly their ability to differentiate between it and *Bacillus influenzae*. No differences in ability to bind complement were observed among twelve typical pertussis strains. Some cross reaction, although weak, was observed in two atypical strains and two strains of hemoglobinophilic bacilli.

The work was done with immune sera produced by the inoculation of rabbits with live cultures of the various organisms. The original

Wassermann technic reduced to one-tenth volume was used. The antigen which was found to give the best results was prepared as follows: A forty-eight hour growth on Bordet-Gengou medium was taken up in neutral distilled water and shaken for three to four hours in an electric shaker, the resulting emulsion allowed to stand at 56°C. over night, filtered through a Berkefeld, and the supernatant fluid used after being rendered isotonic with 9 per cent salt salution.—H. W. L.

Pollen Extracts and Vaccines in Hay Fever. SOLOMON STROUSE and IRA FRANK. Journ. A. M A., 1916, *66*, 712–715.

That pollen is the etiologic agent in hay fever cannot be questioned, but that it is the only factor is not certain.

It is possible that hay fever is a pollenosis associated with bacterial subinfection. It may be that the inhalation of pollen in susceptible individuals irritates the nasal mucosa rendering it more liable to bacterial infection and that this infection in turn favors the absorption of more pollen.

Cultures from the nose yielded in most instances pure cultures of *Staphylococcus albus*, although the pneumococcus and *Micrococcus catarrhalis* were occasionally found. Autogenous bacterial vaccines were prepared from the organisms isolated. Thirteen patients were treated with the bacterial vaccines; of these, 64 per cent showed signs of improvement.

A series of patients treated prophylactically with pollen extract alone showed decided improvement. The administration of vaccines following a previous pollen treatment resulted in seasonal cures. G. H. S.

Equilibrium in the Combination and the Dissociation of Precipitates. RICHARD WEIL. Proc. N. Y. Pathol. Soc., 1915, *15*, 132–134.

If a serum or other similar antigen be mixed with its specific precipitating anti-serum, the resulting precipitate never exhausts completely either of these two factors. Furthermore the serum of immunized animals sometimes contains both precipitin and precipitinogen. When a chemically pure antigen, namely crystallized egg albumen, is mixed with its specific anti-serum, a precipitate forms. The supernatant liquid can always be shown to contain one of the two factors, either egg albumen or antibody, but never both at the same time. Therefore it is concluded that under proper experimental conditions the precipitation reaction goes on to complete exhaustion of one factor and that equilibrium, in the sense of mass action, does not exist. The results of earlier observations are therefore explained by the presence of a multiplicity of antigens and antibodies, as was first suggested by Von Dungern. Furthermore the presence of a third colloid, such as rabbit serum, does not interfere with the completeness of the reaction.

In the subsequent discussion Dr. Weil stated that by heating a precipitin to 72° it is possible to deprive it completely of its precipitating property while the sensitizing value is retained almost unimpaired.— W. J. M.

LABORATORY TECHNIQUE

An Electrical Furnace for Sterilizing Inoculating Loops. H. J. CORPER. Journ. A. M. A., 1916, *66*, 187.

The author describes the construction of an electrical furnace for sterilizing platinum loops.—G. H. S.

Two Laboratory Suggestions. GEO. B. LAKE. Medical Record, 1916, *89*, 422–423.

An eye shade for microscopical work is recommended.

By the addition of a small quantity of acid or alkali, tone may be restored to Wright's stain, which has deteriorated with age.—M. W. C.

A Method of Obtaining Suspensions of Living Cells from the Fixed Tissues, and for the Plating Out of Individual Cells. PEYTON ROUS and F. S. JONES. Proc. Soc. Biol. and Med., 1916, *13*, 73.

Bits of tissue are cultivated in plasma and the growing cultures flooded with trypsin dissolved in Locke's solution. The fibrin network is dissolved and the spherical living cells released. These are washed and plated anew.—W. J. M.

A Simple Method for Blood Cultures. PAUL G. WESTON. Jour. A. M. A., 1916, *66*, 507.

An ordinary vaccine ampule is half filled with culture medium. The neck is drawn to a capillary tube. A vacuum is obtained in the ampule and the capillary tube is sealed. A rubber tube, with needle for insertion into the vein, is placed over the capillary tube. The apparatus is then sterilized.

After puncture of the vein the capillary tube is broken. After the collection of blood no sealing is necessary as a firm clot plugs the needle.—G. H. S.

A Stain for Tubercle Bacilli. EMANUEL KLEIN. New York Med. Jour., 1916, *103*, 217.

The author suggests as a substitute for the usual carbol-fuchsin, acid alcohol, methylene blue stain for tubercle bacilli, the following:

(1) 3 per cent alcoholic solution of crystal violet.

(2) 1 per cent aqueous solution of ammonium carbonate.

(3) 10 per cent solution of nitric acid (C. P.).

(4) 95 per cent alcohol.

(5) Saturated alcoholic solution of Bismarck brown of which enough is added to water to make a tincture of iodine color.

(1) and (2) are mixed in proportion 1:3. This is placed upon smear, which has been fixed in the usual manner, and allowed to steam and cool three successive times. Excess stain is poured off, slide washed in tap water. (3) and (4) are added alternately with rinsing after each, until specimen is perfectly colorless. Without washing, (5) is added for three minutes. Slide is dried and examined. The chief advantage of this stain is the contrast obtained with tubercle bacilli stained violet upon a light brown background.—M. W. C.

MEDICAL BACTERIOLOGY

Present Views in Respect of Modes and Periods of Infection in Tuberculosis. MAZYCK P. RAVENEL. Jour. A. M. A., 1916, *66*, 613.
A general review of the literature on the subject.—G. H. S.

Influenza. A. H. DOTY. Medical Record, 1916, *89*, 455–456.
A general discussion of influenza with special emphasis upon means of prevention. M. W. C.

Chronic Tonsillitis. LOUIS FISCHER. New York Med. Jour., 1916, *103*, 147.
Bacteriological examinations of the throats of cases of chronic tonsillitis have shown the presence of *Staphylococcus aureus*, and an occasional streptococcus, never the Klebs-Löffler bacillus.—M. W. C.

Peritonitis Following Acute Ovaritis of Anginal Origin. RUSSELL M. WILDER. Jour. A. M. A., 1916, *66*, 659.
In the authors opinion many cases of so-called primary peritonitis result from infection of the throat passing to the ovaries and finally causing peritonitis. In the author's case diplococci and streptococci were found.—G. H. S.

Two Unusual Strains of Diphtheroid Bacilli. RALPH R. MELLON. Medical Record, 1916, *89*, 240.
A preliminary note briefly describing the cultural and biological characteristics of two strains of diphtheroid bacilli, both of which are pathogenic for animals. One of the strains is of especial interest culturally because of a most marked pleomorphism.—M. W. C.

Common Affections of the Eye. S. D. RISLEY. New York Med. Jour., 1916, *103*, 145.
Bacteriological examinations of the discharges from a large number of cases of ophthalmia neonatorum indicate that the disease is not always due to the gonococcus, but frequently to a variety of other microorganisms. Gonococcus is present in from 50 to 65 per cent of the cases.—M. W. C.

The Control of Diphtheria Epidemics. W. D. STOVALL. Jour. A. M. A., 1916, *66*, 804–806.
The author reports an epidemic of diphtheria in which the Schick test was employed. The use of the skin reaction and throat swabs together with prophylactic administration of antitoxin where indicated presents a most satisfactory method of combating epidemics of diphtheria.—G. H. S.

Removal of Tonsils and Adenoids in Diphtheria Carriers. S. A. FRIEDBERG. Jour. A. M. A., 1916, *66*, 810.
Report of 6 cases of diphtheria carriers in which the condition could not be remedied by the local application of kaolin.

Removal of the tonsils and adenoid tissue resulted in the prompt disappearance of the organisms upon culture.—G. H. S.

A Study of the Etiology of Chronic Nephritis. P. K. BROWN and W. T. CUMMINS. Journ. A. M. A., 1916, *66*, 793–797.

From the study of a large number of cases of nephritis the authors conclude that venereal and other serious infections, chiefly streptococcus and pneumococcus, have a very definite bearing on the occurrence of advanced kidney disease.—G. H. S.

Experimental Syphilis in the Rabbit Produced by the Brain Substance of the Living Paretic. UDO J. WILE. Jour. Exp. Med., 1916, *23*, 199–202.

Brain tissue from living paretics easily produces experimental syphilis in rabbits and the spirochaetes contained in this living tissue constitute a virulent strain with a shorter period of incubation for the rabbit than exists with other strains.—B. W.

The Incidence of Syphilis Among Juvenile Delinquents. THOMAS H. HARRIS. Journ. A. M. A., 1916, *66*, 102.

Wassermann tests performed on the sera of 365 juvenile delinquents taken without selection, gave positive results in about one-fifth of the cases. The author regards much of the infection as acquired rather than congenital. The relation of syphilitic infection to mental status is discussed.—G. H. S.

Cultural Experiments with the Spirochaeta pallida Derived from the Paretic Brain. UDO J. WILE and PAUL HENRY DE KRIUF. Jour. A. M. A., 1916, *66*, 646.

Rabbit inoculation with paretic brain has yielded pure cultures of pallida. The strains may be cultivated in artificial media. The organisms from brain tissue are morphologically identical with spirochaetes derived from cutaneous syphilids, although the growth of the latter is much more luxuriant.—G. H. S.

Diphtheria Carriers. J. C. GEIGER, FRANK L. KELLY, and VIOLET M. BATHGATE. Jour. A. M. A., 1916, *66*, 645.

Nose and throat cultures were taken from all contacts in six investigations. Nose cultures gave 42.2 in the percentage average of positives, throat cultures 7.9. Of all positive cultures 72 per cent were nose and 28 per cent throat.

The Schick test applied in one investigation proved of value in distinguishing between contacts and carriers.—G. H. S.

Rôle of the Lymphatics in Ascending Renal Infection. DANIEL N. EISENDRATH and JACOB V. KAHN. Jour. A. M. A., 1916, *66*, 561.

In a series of experiments on dogs and rabbits the authors have demonstrated that infection of the bladder with the *Bacillus coli,*

Staphylococcus aureus and *Proteus vulgaris* may result in infection of the kidney with these organisms.

Infection travels by way of the lymphatics in the wall of the ureter and not along the mucous membrane.—G. H. S.

The Etiology of the Current Epidemic of Respiratory Infections in Chicago. GEORGE MATHERS. Jour. A. M. A., 1916, *66*, 30.

Cultures obtained from the sputum, nasal discharge and the pharyngeal mucosa of twenty-four cases of respiratory infection revealed the fact that in seventeen instances the predominating organism was a hemolytic streptococcus, culturally resembling the *Streptococcus pyogenes* type. Pigment-producing streptococci and pneumococci were uniformly found. *B. influenzae* and *M. catarrhalis* were not present in any of the cultures.—G. H. S.

The Cause of Rat-Bite Fever. KENZO FUTAKI, ETSUMA TAKAKI, TENJI TANIGUCHI, and SHIMPACHI OSUMI. Jour. Exp. Med., 1916, *23*, 249–250.

A preliminary note in which is announced the finding of a spirochaete in the skin and in a lymph gland of patients suffering with rat-bite fever. The skin tissue and blood drawn from a patient when injected into monkeys, guinea pigs and white rats produced infection and the disease could be transmitted from these to other animals. Further details are promised.—B. W.

The Etiology of Rat-Bite Fever. FRANCIS G. BLAKE. Jour. Exp. Med., 1916, *23*, 39–60.

A case of rat-bite fever coming under the author's observation, terminating fatally and coming to autopsy, afforded an excellent opportunity for studying the etiology of this disease. An organism, which the author identifies as the *Streptothrix muris-ratti*, was demonstrated in a mitral vegetation and isolated in pure culture from the blood. The patient's serum contained strong agglutinins for the *Streptothrix*.—B. W.

Influenza and Grippe in Infants and Children. CARL G. LEO-WOLF. Medical Record, 1916, *89*, 226.

A discussion of influenza and grippe in children. All phases of the subject, such as history, etiology, pathology, treatment, etc., are treated in detail.

Emphasis is placed upon the fact that the two diseases are manifestly distinct entities. Both are due to bacterial infection, influenza being caused by the cocco-bacillus of Pfeiffer, grippe by one or more of a number of bacteria-pneumococcus, *Micrococcus catarrhalis*, bacillus of Friedländer, streptococci, and bacteria living as saprophytic parasites in the mouth.

In both influenza and grippe particular stress should be laid upon prophylaxis.—M. W. C.

Rocky Mountain Spotted Fever. HENRY C. MICHIE, JR. and HOUSTON
H. PARSONS. Medical Record, 1916, *89*, 266.

A comprehensive investigation of Rocky Mountain spotted fever,
which includes a résumé of all work done upon the disease as well as an
account of the results of an extensive research on the fever as it occurs
in the Bitter Root Valley, Montana. The report gives in detail the
history, geographical distribution, etiology, symptoms, pathology, diag-
nosis, treatment, prophylaxis, prognosis, and epidemiology of the
disease.—M. W. C.

Remarks on B. Welchii in the Stools of Pellagrins. W. H. HOLMES.
Arch. Int. Med., 1916, *17*, 453–458.

In a study of the stools of pellagrins an abnormally large number of
organisms of the *B. Welchii* group have been found. These organisms
are able to produce diarrhea in the presence of a high carbohydrate
diet, which can be cured by the substitution of a protein diet. Since
he believes that pellagra is caused by a high carbohydrate diet, the
writer suggests a further investigation of the rôle of *B. Welchii* in this
disease.—G. H. R.

The Treatment of Infections of Accessory Sinus. A. M. MACWHINNIE.
New York Med. Journ., 1916, *103*, 213.

Theoretically, the treatment of ethmoiditis should consist in the ad-
ministration of an autogeneous vaccine made from all types of bacteria
isolated. In most cases these vary in number from three to five. The
use of such a vaccine has met with marvelous results in a few cases,
but the average of successful treatments is 30 per cent.

The author recommends a system of cleansing to be used in con-
junction with the application of his suction pump.—M. W. C.

Salvarsan in Primary Syphilis. ALEXANDER A. UHLE and WM. H.
MACKINNEY. New York Med. Jour., 1916, *103*, 6.

Treatment of primary syphilis with salvarsan is most successful in
cases in which a diagnosis is established sufficiently early to allow
prompt administration of the drug. The earliest positive diagnosis of
syphilis can be made by examination by dark field illumination of the
expressed serum of a suspected sore. A Wassermann reaction is not
positive until the seventh to fourteenth day after the appearance of the
chancre. The Wassermann reaction is of value in the diagnosis of
syphilis, as a positive reaction means the onset of systemic syphilis,
while a negative reaction, for practical purposes, means a local infection
only.—M. W. C.

The Prompt Cure of Gonorrhea. GEORGE A. WYETH. New York Med.
Jour., 1916, *103*, 244.

Treatment with a 0.25 to 0.5 per cent solution of protargol, if begun
within twenty-four hours after the appearance of a purulent discharge,

has resulted in a cure within five to seven days in 60 per cent of the author's cases of gonorrhea.

New, well developed cases, where no evidence of phagocytosis is shown, are more stubborn in yielding to treatment than cases where the gonococci are mostly intracellular. In the latter group of cases, the use of vaccines is indicated.—M. W. C.

A Study of the Bacteriology of Chronic Prostatitis and Spermatocystitis. HARRY B. CULVER. Jour. A. M. A., 1916, *66*, 553.

Review of literature. Detailed account of technic employed by the author.

34 cases examined organisms were recovered from 70 per cent. Twelve different organisms were isolated—*Staphylococcus albus, Streptococcus hemolyticus, Gonococcus,* diphtheroid bacillus, *Micrococcus tetragenus* and *M.catarrhalis, Bacillus proteus,* a colon-like bacillus, anaerobic staphylococus and streptoccccus and unidentified gram-negative cocci and diplococci.

Skin tests, agglutination and opsonic determinations showed that in 66 per cent of the cases tested the organisms isolated appeared to be specific for the infected individual. Vaccine treatment was apparently beneficial.—G. H. S.

Gallbladder Diseases. C. H. MAYO. New York Med. Jour., 1916, *103*, 433–436.

Diseases of the gallbladder are of infectious origin. Cultures made from the tissues of actively diseased gallbladders. and inoculated intravenously into experimental animals caused disease of the gallbladder, even to occasional stone formation, in 61 per cent of 41 animals. (Rosenow.)

Stones removed from the gallbladder may retain living bacteria for years. The stone is the result of the infection, not the cause of the disease.

The mode of infection is not yet known. Several theories have been advanced, the most probably being that of Rosenow—that the tissues of the gallbladder are open to infection through the vascular system. Typhoid bacteremia is frequently the etiological factor and in this case the attack is undoubtedly through the vascular system.

Several methods of treatment are described.—M. W. C.

The Bacteriology of the Recent Grip Epidemic. CHARLES HALPIN NAMMACK. Medical Record, 1916, *89*, 369.

Cultures made from 50 cases, which had been clinically diagnosed as grip, revealed the following findings:

Influenza-like bacilli in 19 cases, in 6 cases alone and in 13 associated with other microörganisms.

Pneumococcus	in 13 cases
Hemolytic streptococcus	in 6 cases
Friedlander's bacillus	in 3 cases
Staphylococcus	in 3 cases
Micrococcus catarrhalis	in 1 case

These organisms were isolated by means of sputum and nasal cultures.

Two cases are reported in which after recovery there were isolated pure cultures of capsulated pneumococci in one and *Bacillus influenzae* in the other. Such findings emphasize the necessity of taking precautions against infection from persons who are undoubtedly carriers of organisms that may cause grip.—M. W. C.

So-Called Grippe. J. B. RUCKER, JR. New York Med. Jour., 1916, *103*, 294.

Bacteriological examinations of 20 cases of so-called grippe presented findings as follows: 8 containing pneumococcus, typical at least in morphology; 6 containing atypical pneumococcus or *Streptococcus mucosus*; 20 containing small gram positive biscuit-shaped diplococci; 2 containing the bacillus of Pfeiffer, in smear only.

Of all organisms isolated, pathogenicity for animals was established only with the small gram positive diplococci. These organisms caused death of mice in 2 cases, and typical grippe-like symptoms in a rabbit in 1 case. Results of examination of these 20 cases suggest that the etiological factor in the recent epidemic of so-called grippe is the small gram positive diplococcus isolated from all cases.—M. W. C.

Routine Wassermann Examinations of Four Thousand Hospital Patients. I. C. WALKER and D. A. HALLER. Jour. A. M. A., 1916, *66*, 488.

Routine examinations of 4000 hospitals admissions were made. The prevalence of unsuspected syphilis and the frequency of positive reactions in various diseases were studied.

The reaction was positive in 600 cases. Of the 600 positive patients 48 were in the very early stages of syphilis, 306 were in a later stage, and 120 were in cases having involvement of the central nervous system. 13 cases were congenital.

There were 54 positive cases with aortic disease, 10 with epilepsy, 10 with disease of the liver, 10 with disease of the kidney, 9 with pneumonia, 7 with diabetes, and 13 distributed among miscellaneous diseases.

The authors conclude that syphilis is more prevalent than is ordinarily supposed and that infectious diseases, such as typhoid fever, pneumonia, tuberculosis and scarlet fever, do not cause false positive reactions.—G. H. S.

The Treatment of Gastric Ulcer. A. F. R. ANDRESEN. Medical Record, 1916, *89*, 457–459.

A rational, specific therapy of gastric ulcer should be based upon the fact that this pathological conditions is due to an infective process, the etiological agent of which is *Streptococcus viridans*.

Treatment in cases of simple ulcer should consist first of removal of the cause of infection, second of rest of the infected part, and third of efforts to overcome the infection and to repair the injured tissues.

Under the third heading, the use of autogenous vaccines is of chief importance. Such vaccines have proved of great assistance in clearing up foci of infection, as well as in exerting a beneficient action on ulcer symptoms. Vaccines were used by the author in 38 cases with constantly successful results.—M. W. C.

The Treatment of Diphtheria Carriers with Iodized Phenol. W. O. OTT and K. A. ROY. Jour. A. M. A., 1916, *66*, 800–802.

The treatment of 17 diphtheria carriers by the use of iodized phenol is reported.

The solution (60 per cent phenol, 20 per cent iodine crystals and 20 per cent glycerin) was swabbed over the tonsils, uvula and posterior wall of the pharynx in pharyngeal cases and over the entire anterior part of the nasal cavity in nasal cases. Applications were made every 48 hours until negative cultures were obtained.

No bad results were noted from the use of the preparation although the application is painful for half a minute or less until the anesthetic action of the phenol takes effect.

Negatives cultures were obtained in 35 per cent of the cases after one application; in 29 per cent after the second; in 12 per cent after the third; in 6 per cent after the fifth and in 12 per cent after the sixth. One case required 9 applications.

Fifteen cases were followed from one to three weeks after leaving the hospital and yielded negative cultures.—G. H. S.

The Extent and Significance of Gonorrhea in a Reformatory for Women. ELIZABETH A. SULLIVAN and EDITH R. SPAULDING. Journ. A. M. A., 1916, *66*, 95.

An exhaustive study of 500 women delinquents with respect to the prevalence of gonorrheal infection; the nature, duration and extent of the infection, together with its susceptibility to treatment; the effect of the infection in producing other pathologic conditions; its relation to birthrate; and general considerations of an economic and sociologic nature.

Among 522 cases examined, 395 or 75.7 per cent were found to be gonorrheic. The average duration of infection was 4 years, 5 months, the case of longest duration being 26 years. In 82.7 per cent of the cases there had been no cessation of clinical symptoms since the initial infections. With respect to treatment during the course of the infection, the clinical history of 378 cases showed that but 1 per cent had received adequate medical treatment.

A comparison of the birthrate among gonorrheic and non-gonorrheic women showed that the average number of children among the former was 1.1, among the latter, 2.8.

The amount of treatment required to eradicate the clinical symptoms depends on the duration of the disease previous to treatment. After disease has lasted from 4 to 6 months without treatment, 10 to 12 months' treatment is usually necessary.—G. H. S.

The Epidemiology of Tuberculosis. F. C. SMITH. Journ. A. M. A., 1916, *66*, 77.

A general discussion of the subject.

The climate and topography of the country cannot of themselves constitute immune zones. Such areas are simply uninfected territory.

Infection of a majority of all persons occurs before the age of 12 years. Such factors as street dust, flies, water and fomites are probably of less moment in causing infection then direct contact. The lymph glands as avenues of infection are significant.

The importance of infection with the bovine type is indicated by the fact that 8 per cent of deaths from tuberculosis are due to this agent.

While it is granted that certain occupations predispose to tuberculosis and that age, social condition, economic state, and race may be potent factors, It is most certain that physical exhaustion, whatever may be its cause, entails the failure of some of the natural defenses, and latent infection becomes active.

Gross infection should be avoided; a diagnosis should be made at the earliest possible time, but in the eradication of tuberculosis the greatest problem is the economic one.—G. H. S.

Tuberculosis. HERMANN M. BIGGS. New York Med. Jour., 1916, *103*, 168.

In reviewing the progress made in the treatment and control of tuberculosis during the past 20 years, the author states that while much has been done toward eradicating the disease, researches upon tuberculosis have not added anything essentially new to the knowledge of the subject. A clearer definition has been given to certain phases of the disease, such as the establishment of the facts that bovine infections play practically no part in the production of pulmonary tuberculosis, but do cause 30–35 per cent of the tuberculosis of lymph nodes of children under five years; that pulmonary tuberculosis is practically always the result of the direct transmission of tubercle bacilli from the sick to the well; and that the disease is definitely preventable.

Neither a specific treatment nor an effective method of producing insusceptibility for tuberculosis has been discovered. Tuberculins and various forms of modified vaccines are receiving less recognition than formerly. The evidence of the wide dissemination of tuberculous infections in early life renders the use of tuberculin of little value as a diagnostic agent.

With the use of the X-ray, some progress has been made in diagnosis and with the aid of an earlier diagnosis a larger per cent of recoveries is probable.

As a constructive program for further eradication of tuberculosis, emphasis should be laid upon extensive improvements in preventive measures. Among these are disinfection of tuberculous material, caution in disseminating the disease, increased facilities for bacteriological diagnosis, adequate provision for institutional care of tuber-

culous cases, the extension of nursing service of the type now done by the visiting public health nurse, insistence upon pasteurization of milk supplies, and vigorous prosecution of the educational compaign.—M. W. C.

The Period of Life at which Infection from Tuberculosis Occurs most Frequently. S. ADOLPHUS KNOPF. Medical Record, 1916, *89*, 47.

A study of several still unsolved problems of tuberculosis brings forth the following facts, based upon the opinions and statistical evidences of a large number of authorities upon tuberculosis and children's diseases.

Tuberculous diseases in childhood, compared with tuberculous infection, is relatively rare (36 per cent). Tuberculous infection in infants and young children is exceedingly frequent and the majority of cases in the adult can be traced to a childhood infection. Such an infection is most apt to become active about the fifteenth year; if not then, between 18 and 30. A tuberculous infection contracted in later life usually occurs between the ages of 20 and 35. It is probable that prenatal infection is more frequent than has been generally believed. The frequency of infection increases with the age of the child and is affected by environment.

Lungs and lymph nodules are the organs most frequently involved in children; secondly, bones; thirdly, intestines; and fourthly, meninges.

The most common sources of infection are contact with tuberculous individuals and infected food; especially milk from tuberculous cows.

The most successful means of combating tuberculosis is to diminish the source of infection in childhood. In order to do this, there must be a radical change in our present regulations in regard to the disease and a much more extensive provision for the care of the infected. Particular attention should be given to preventive measures, especially the establishment of an extensive educational system and the improvement of social conditions. These changes can be best accomplished by a Federal Commission on Tuberculosis.—M. W. C.

The Epidemic of Typhus Exanthematicus in the Balkans and in the Camps of Europe. BERT. W. CALDWELL. Jour. A. M. A., 1916, *66*, 326.

A general discussion of the epidemic, its causes and extent; and the means employed in its control.

One person out of every five of the population was attacked by typhus, the fatal cases numbering 135,000. The hospital mortality ranged from 19 to 65 per cent. Conditions (cold weather and congestion of population) peculiarly favorable to the distribution of the disease obtained.

The body louse is a certain, and the head louse a probable, agent in its transmission. Evidence of any other mode of transmission is entirely lacking. With proper hygienic precautions non-immunes are practically safe from infection. The incubation period of the disease

is about fourteen days. Eruption follows the onset closely and reaches its maximum intensity on the fifth day. It is during this five-day period that there is greatest danger of infection. The disease seems to be a general septicemia, the only discovery relative to its pathology being the recovery from the spleen of an organism resembling the Plotz organism.

In the eradication of the epidemic the American Red Cross Sanitary Commission employed such measures as fumigation of all hospitals, barracks, schools and other foci of infection, bathing patients and sterilizing their clothing, maintaining quarantine of patients, and the institution of measures of general sanitation.

The treatment of typhus fever is unsatisfactory and is supportive and symtomatic in character. The serum prepared by Nicolle, or the vaccine prepared from the Plotz organisms tends to abort the disease and apparently has therapeutic value. The prophylactic value of the Plotz vaccine is problematic.—G. H. S.

PROTOZOA AND OTHER ANIMAL PARASITES

Trichinosis. ARTHUR R. ELLIOTT. Jour. A. M. A., 1916, *66*, 504.

A case of trichinosis is reported from which actively motile trichina larvae were found in the spinal fluid.—G. H. S.

Filaria Sanguinis Hominis. CODIS PHIPPS. Journ. A. M. A., 1916, *66*, 266

The author reports a case of infection with *Filaria sanguinis hominis* (*Filaria nocturua, Filaria bancrofti*) in which a cure was effected by the administration of salvarsan.—G. H. S.

Dermatitis Herpetiformis. M. F. ENGMAN and ROBERT DAVIS. Jour. A. M. A., 1916, *66*, 492.

It is probable that the endameba is an etiologic factor in a certain percentage of cases of dermatitis herpetiformis.

In such cases the administration of emetin hydrochloride has proved of value.—G. H. S.

Trichiniasis. MICHAEL G. WOHL. Medical Record, 1916, *89*, 98.

A general review of the disease with the report of one case.

In discussing methods of treatment, the author states that the administration of vaccines prepared from trichinae derived from infected hogs would be a logical step, as specific antibodies have been demonstrated in the blood of patients suffering from trichiniasis.—M. W. C.

Thionin as a Diagnostic Stain for Pyorrhea Alveolaris. MARTIN DUPRAY. Jour. A. M. A., 1916, *66*, 507.

An excellent diagnostic stain for endamebae may be prepared as follows:

Thionin	0.5 gm.
Distilled water	100.0 cc.
Phenol crystals	2.0 gm.

The smear is air-dried, fixed in flame, stained for a few seconds while warm, washed and dried.

The cytoplasm of the endamabae stains a light purplish violet, the nuclei a deeper reddish violet. Ingested blood corpuscles are nearly black. Pus cells are a light blue. Bacteria are well stained, the fusiform bacilli and spirilla being especially plain.

The stain deteriorates in three to four months.—G. H. S.
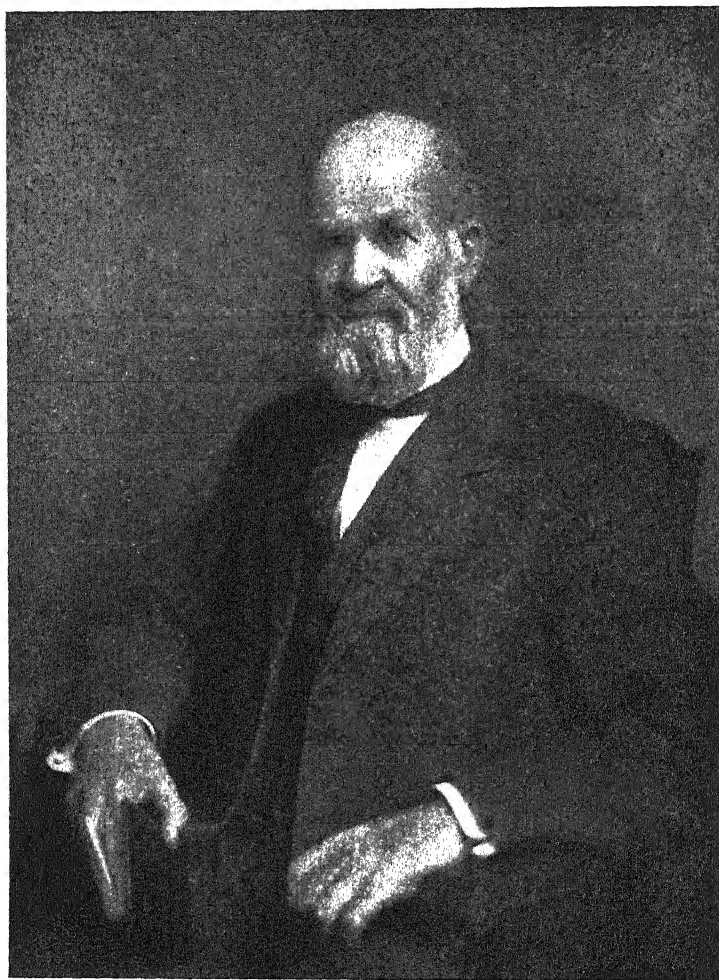
THOMAS JONATHAN BURRILL
[1839–1916]

IN MEMORIAM THOMAS J. BURRILL

ERWIN F. SMITH

In the recent death (April 14) of Prof. Thomas J. Burrill of the University of Illinois, there passed away, at a ripe old age but still in possession of all his faculties, a lovable man of uncommon personality, and one who contributed materially during his earlier years to the advancement of American science. In America we have a peculiar way of treating all those who have demonstrated the possession of research ability of a high order, which may be designated as a method of extinction by promotion. As soon as a man becomes conspicuous through his researches, boards of control find other things for him to do, more in keeping with their ideas of efficiency and eternal fitness, and he ceases to contribute further, except perhaps very indirectly, to the advancement of science. Professor Burrill was no exception to this rule. He never lost his interest in science and having a high order of mind he was peculiarly fitted to be a productive research worker, but from middle life on it was his misfortune, recognized by him as such, but borne with cheerfulness, to have his time absorbed by administrative duties connected with his university, of which he was at one time the acting head. His actual contributions, however, were amply sufficient to perpetuate his memory.

In addition to his mycological studies, which he pursued with great eagerness and with good results ("Fungi of Illinois") he studied the bacteria at a time (1870–1882) when literature was scanty, methods were crude, and microscopes were not what they are today. Into this field of darkness, or at best of dim half-lights and perplexed gropings, which he has described to me in memorable words, Burrill projected his keen intellect and brought forth the beginnings of a whole new science, i.e., he discovered and demonstrated in "pear blight" the first bacterial disease

of plants. To Burrill and America belongs this honor, whatever other honor belongs elsewhere! Just as Pasteur's contribution to science is more vital than Koch's, because it was earlier and was pioneer work, so Burrill's discovery was more difficult to make and hence more worthy of praise, than anything that has come after. Anyone of ordinary capacity can follow a blazed trail, but only a great man can hew a path through the unbroken wilderness to be a highway for all men who come after!

Burrill did not publish on pear blight fully, in the modern sense of that word, for he was a pioneer, but in studying the freshly diseased tissues (and he had the wisdom to select just those) he saw clearly in many sections that fungi were not there and that swarms of bacteria (called by him *Micrococcus amylovorus*) were always present and were therefore probably the cause of this mysterious disease. Acting on this assumption he took masses of these bacteria which his microscope had shown to be free from fungi (with a multitude of whose forms he was already very familiar) and with them by inoculation reproduced the pear disease, not once but many times. Others, elsewhere, in these same early days made similar announcements, but were less fortunate or less painstaking, since no one in later days has been able to confirm their findings, whereas Burrill's discoveries have been confirmed a hundred times, and relate to one of our most serious orchard diseases, known for a hundred years, and for the control of which the nation and the orchard states are still spending much time and money.

Professor Burrill was born at Pittsfield, Mass., April 25, 1839. He was educated at the Illinois State Normal School, and was always a teacher, and a good one. He held honorary degrees from the University of Chicago (Ph.D., 1881) and The Northwestern University (LL.D.,1893), and was a member of various scientific societies. I remember seeing him first at meetings of the American Association for the Advancement of Science, of which he was long a member, and this year president of Section G (Botany). He had also been president of the American Microscopical Society, 1885–86, and was president of the Society of American Bacteriologists at the time of his death.

Professor Burrill was very companionable and very helpful to his students. He was also much respected by his colleagues the country over. At one of the last scientific gatherings he attended (the twenty-fifth anniversary of the Missouri Botanic Garden held at St. Louis in 1914), when his name was incidentally mentioned by one of the speakers there was a round of applause from the crowded room. I did not see him after this time but he was then (at 75) very well preserved and intellectually keen.

The ancient Greeks had a proverb "Let not a man boast that he has had a happy life until the day of his death." Professor Burrill would not have boasted of anything since he was quiet and unassuming rather than loud and aggressive, but it may be said for him, that he represented the best type of scientific mind and now that he has gone we may say as we close the ranks and turn away: Happy was this man because he lived unobtrusively, serenely and usefully, and because he died full of years and of honor, loved by all his intimates, and respected by all who knew him.

RESOLUTIONS ADOPTED AT THE URBANA MEETING OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS IN REGARD TO THE WORK OF PROFESSOR BURRILL.

WHEREAS, It is rarely possible for a scientist to make a discovery of such fundamental importance that it serves to develop an entirely new branch of science, and

WHEREAS, One of our hosts at this time, Dr. T. J. Burrill, made such a discovery when he worked out the cause of pear blight and thus founded the science of bacterial plant pathology,

Therefore, be it resolved, that the Society of American Bacteriologists regards it as a peculiar privilege to congratulate him for his pioneer and epoch-making work, and expresses its appreciation of his vigorous enthusiastic and inspiring address of welcome.

Be it further resolved, That a copy of these resolutions be engrossed, signed by the officers of the Society, and presented to Dr. Burrill.

STUDIES ON AEROBIC SPORE-BEARING NON-PATHO-GENIC[1] BACTERIA

PART I

From the Laboratory of Hygiene and Bacteriology, Johns Hopkins University

INTRODUCTION

BY W. W. FORD

One of the most important problems of modern hygiene is the identification and classification of the bacteria in our environment. Microörganisms of various kinds exist everywhere in nature and influence profoundly all sorts of substances which affect man's physical condition. This is true of food-stuffs in general and especially true of milk which is markedly altered in its chemical composition by the bacteria which multiply in it. The microörganisms in our environment are of various sorts, pigmented bacteria, spore-bearing bacteria, yeasts, moulds, etc. Some of these forms are identified without great difficulty but our knowledge of the spore-bearing bacteria is still in a state of chaos. The reason for this lack of knowledge is not far to seek. The science of bacteriology developed primarily among physicians whose interest naturally lay in the disease-producing properties of the various parasites which infect man and the animals. Non-pathogenic bacteria were of importance chiefly as laboratory contaminations to be avoided. With the development of industrial bacteriology those species were again most carefully studied which seemed to serve some distinct purpose in nature, as for example, the nitrifying bacteria of the

[1] The term "non-pathogenic" is used here in the sense of "lacking in disease-producing properties." Many spore-bearing bacteria are at times pathogenic to small animals and instances are reported in which they may produce inflammatory reactions when vegetating on mucous surfaces. The organisms here described are however in no instances capable of producing definite diseases

soil and the lactic acid bacteria in milk. In consequence the bacteria found in nature which seem to be lacking in any definite function have been largely neglected. At various times many species of spore-bearing organisms have been described and recorded in the literature and in many instances these cultures have been kept alive in laboratories both in Europe and in America. It would seem an easy task therefore to collect the spore-bearing bacteria from different institutions, make a careful study of their properties and arrive at some conclusion as to their identity and classification, just as is done with the pathogenic species. This method of solving the difficulty is open to serious objections, however, and has not thus far proved of great value. In the first place the descriptions originally given of many of these species are meager and the original cultures have not been saved. In consequence the literature of bacteriology is thickly strewn with names of spore-bearing organisms which have absolutely no meaning. The term *Bacillus subtilis* for instance is applied to almost any large microorganism which forms spores readily and grows abundantly on artificial media, and cultures identified as *Bacillus subtilis* by different bacteriologists are often found to have little or nothing in common. Again the cultures which have been kept alive have in many instances so changed in character as no longer to give the reactions orginally described. Thus Migula (1897) found that of some six hundred cultures obtained by him from the laboratories in Germany only a small number had the characteristics first ascribed to them. Finally, pure strains of spore-bearing bacteria are more difficult to keep in direct descent in the laboratory than are other species. When cultures become contaminated it frequently happens that the contaminating species is picked up from the plates made to purify the strain and carried on as the original. This has happened a number of times in our own laboratory during the past few years and in consequence we have become very sceptical of the value of any conclusions based upon a comparison of existing stock cultures.

A number of years ago an attempt was made, in the laboratory of Dr. Adami in Montreal (Ford, 1903) to separate and classify

the sporulating organisms by the use of carbohydrates.   The result of this work was not entirely satisfactory because of the difficulty of establishing the fundamental species from which to build up our system of classification.   During the past few years, however, a number of very valuable papers on spore-bearing bacteria have appeared in the literature and have cleared up some of the most difficult points.   Of especial importance is the work of Meyer (1903) and his collaborators, Gottheil (1901) and Neide (1904) in Germany, and the work of Chester (1903) in this country.   As a result of the efforts of these authors we now have accurate descriptions and definite means of identification of a small number of our most common spore-bearing species.

Some four years ago a large number of spore-bearing bacteria was obtained from raw milk and from milk heated to various temperatures from 60° to 100° and so much difficulty was encountered in their identification that it seemed as if the time was ripe for a more extensive investigation of the subject, based upon the work above referred to.   The problem was first undertaken by Mr. Lawrence and myself with the organisms from milk.   After a working basis had been obtained for the classification of these species a study of the spore-bearing bacteria of water was undertaken by Dr. Laubach, and of the soil by Dr. Laubach and Mr. Rice with the object of testing the classification already adopted for milk bacteria and of adding to it such species as had not previously been encountered in our work. Finally stock cultures of well-known species were obtained from the Kral collection in Vienna, the Winslow collection in the American Museum in New York, from the laboratories of hygiene of the University of Pennsylvania, and of the University of Chicago, and the bacteriological laboratory of the Sheffield Scientific School, and our cultures were compared with them. From the start of the work however, our object has been to establish clearly the different types of spore-bearing organisms in our own laboratory and then to link these types up with types already established by other observers.   Altogether over 1700 cultures have been studied from various sources, milk, soil, dust,

water, intestinal contents and contaminated plates. From this number we have obtained 28 distinct types of which 22 are clearly to be identified as well known species, 2 are distinct · varieties of old types and 4 are evidently new species. In general our aim has been to clarify our knowledge in regard to old species and not to establish new types except when our isolations showed certain characteristics not already referred to in the literature and of distinguishing importance.

The media employed in this work were the standard media of the laboratory. A great deal of emphasis was laid upon the reactions with gelatin, with litmus milk, with glucose, saccharose, and lactose broth, with glucose litmus agar and with Loeffler's blood serum. The morphology was studied from smears made from plain and glucose agar cultures 6 to 8 hours old and 22 to 24 hours old, and from cultures 1 to 2 weeks old, the organisms being always stained with Gentian violet. The same preparations were used later for measurements and for illustrations. The method of sporulation and the size, shape and position of the spore were observed with great care. A study of the spore wall, and its differentiation into the exine and intine of Gottheil and Chester, while interesting and important, proved of little help in classification. The method of spore-germination was likewise found relatively valueless. Nearly every type of spore-germination could eventually be found with most species and our observations were so inconstant as not to furnish any basis for classification. Micro-chemical reactions, while undoubtedly of great value, could not be worked out with any degree of thoroughness and were eventually discarded. Careful observations were made upon the thermal death points which were established with broth cultures subjected to various degrees of temperature in the Arnold sterilizer and in the autoclave. In general our classification may be said to rest upon morphological and tinctorial properties, spore-formation and cultural reactions. How valuable our results are can only be determined by the extent to which other workers may be able to utilize this classification in subsequent investigations.

SPORE-BEARING BACTERIA IN MILK

BY J. S. LAWRENCE AND W. W. FORD

It has been pointed out by a number of observers (Flügge, 1904; Ford and Pryor, 1904) that milk heated to any temperature above 60°C, if kept in a warm place, shows an excessive development of spore-bearing bacteria which are ordinarily inhibited by the lactic acid bacteria universally present. Hueppe (1884) was possibly the first to call attention to the presence of aerobic spore-bearing forms in milk but it is impossible to say now what his *Bacillus butyricus* (an aerobe) really was. Subsequently Loeffler (1887) described an organism from boiled milk which had been allowed to clot, under the name *Bacillus lactis albus*, now known as *Bacillus albolactus* Migula. Considerably later Flügge (1894) took up the question at some length and described eleven different species found in boiled milk and to them he ascribed an etiological rôle in the summer diarrhoea of infants. Several of the organisms described by Flügge are now considered identical with such common saprophytes as *Bacillus vu'gatus* and *Bacillus mesentericus* while others can be identified with difficulty or not at all, as their originals have been lost. During the past three years we have worked out the morphological and cultural reactions of 250 spore-bearing bacteria obtained from raw milk and from milk subjected to various temperatures. The two most common species proved to be *Bacillus cereus* of Frankland and *Bacillus subtilis* of Cohn. In this differentiation we follow Chester who has given us a definite and clear conception of Cohn's species and has taken up at length the somewhat involved discussion concerning the two organisms. As a result of his work Chester decided that the real *Bacillus subtilis* of Cohn is one of the smallest of the spore-bearing species, forms central or slightly excentric spores which have a characteristic appearance and gives definite cultural reactions. The reactions as outlined by Chester we are able to confirm in the main but we disagree absolutely from him in his contention that this species is identical with *Bacillus vulgatus*, (*B. mesentericus vulgatus*) the old fashioned "potato bacillus."

The cultures identified by us as *Bacillus subtilis* corresponded in a'l particulars to a culture sent us several years ago by Chester and kept in the laboratory since then.   The particular points by which *Bacillus subtilis* may be differentiated from *Bacillus vulgatus* are the development on glucose litmus agar where it forms a dry hard warty growth made up of dense masses of material clinging firmly to the medium, in which may be observed numerous blebs or blisters containing milky fluid, and on Loeffler's blood serum where a similar growth appears, often however with a distinct red color.   On both glucose litmus agar and blood serum *Bacillus vulgatus* develops as a soft wrinkled friable mass easily broken ánd lifted from its substratum.   On potato the *subtilis* differs from the *vulgatus*. The former produces at first a rather dense whitish or greyish mass often showing blebs similar to those on agar and blood serum, a distinct red line appearing in the potato a little distance from the growth, from which characteristic the name *Bacillus subtilis-ruber* is frequently employed.   After 48 to 72 hours a wrinkling appears, the growth later becoming moist and homogeneous.   *B. vulgatus* produces a wrinkled growth from the start, this becoming extremely abundant in 3 to 4 days and frequently assuming a decided pink color.   The differences between the two species are somewhat difficult to describe but when potato cultures of the organisms are placed side by side the points of differentiation become clear and definite.   In general the *subtilis* cultures are dry and hard on solid media and produce firm tenacious scums on fluids, while the *vulgatus* cultures are soft and mealy and their scums friable and easily broken. On a morphological basis it is extremely puzzling to attempt the differentiation of the two types.   In general the rods of *Bacillus vulgatus* are longer and thinner than those of *Bacillus subtilis* while the spores are flatter and bulge the organism only a little if at all.

This view of *Bacillus subtilis* of Cohn and the interpretation put on Cohn's work by Chester is not entirely accepted by bacteriologists but we feel convinced of its correctness except in regard to the differentiation from *Bacillus vulgatus*

already mentioned. The organisms frequently regarded as *Bacillus subtilis* which are characterized by their greater size, their soft mealy growths on hard media, and their thick friable scums we agree with Chester in referring to the "cereus" group the principal type of which, *Bacillus cereus*, was first described by the Franklands. There are two strains of *Bacillus cereus* differentiated by their action on saccharose but it does not seem wise at the present time to divide the species. Our identification of *Bacillus cereus* rests upon Chester's work and upon cultures sent us by him several years ago. In addition a number of strains of *Bacillus cereus* have been received from American laboratories and from the Kral collection in Vienna, all of them agreeing with Chester's in their reactions and thus furnishing us a distinct type by means of which our own strains were identified. *Bacillus cereus* is the most widely distributed aerobic spore-bearing organism in nature in Baltimore and vicinity, as it seems to be in other localities, and possibly has more synonyms than any other species. With these types of *Bacillus subtilis* and *Bacillus cereus* clearly outlined the task of identifying the other spore-bearing organisms became somewhat simpler. *Bacillus vulgatus* was soon found so frequently as to enable us to recognize it without difficulty. One strain of this organism was obtained from the Winslow collection in New York. When freshly isolated the *vulgatus* is very characteristic and differs entirely from other species. The strains isolated in Baltimore were identical with the organisms found in Montreal several years ago and regarded there as *Bacillus vulgatus* and give reactions ascribed to the widely distributed "potato bacillus." *Bacillus mesentericus* (*B. mesentericus fuscus*) was recognized by its morphology and its cultural reactions. In this species we follow Chester. In one instance we obtained a stock culture of *Bacillus mesentericus* which gave the correct reactions as outlined by Chester, this culture coming from the laboratory of hygiene of the University of Pennsylvania. *Bacillus pumilus* of Gottheil we do not regard as a distinct species. *Bacillus aterrimus* (*B. mesentericus niger*) was identified by its production of a black or grey-black pigment, its cultural reactions resembling those of

B. vulgatus. Another organism producing a black pigment and evidently belonging to the mesentericus group was sent us by Winslow as *Bacillus lactis-niger.* It corresponds culturally to *Bacillus mesentericus.* It was not encountered in our work but is included here for the sake of completeness. The same holds true of the organism described originally as *Bacillus mesentericus-ruber* (properly *B. globigii*) a culture of which was obtained from the Kral collection in Vienna. Evidently this is an extremely rare organism in this country as it was never obtained in Baltimore either from milk or from any other source.

One of the most difficult organisms to identify was a species frequently isolated in Baltimore from milk which after boiling clots and peptonizes. In morphology and in its chief cultural reactions it corresponds closely to *Bacillus cereus* but is differentiated from this species by its acid fermentation of lactose and its coagulation of milk. This organism was evidently first described by Loeffler in 1887 as *Bacillus lactis albus (Bacillus albolactus* Migula). We have been unable to obtain a culture of Loeffler's organism but in his original description Loeffler differentiates this species clearly from several other organisms found in milk particularly the ones now known as *Bacillus vulgatus* of Flügge, *Bacillus liodermos* of Flügge, and *Bacillus butyricus* of Hueppe. Since Loeffler was the first to call attention to the presence of an organism in boiled milk which acidifies and clots it and which he differentiated from other spore-bearing bacteria, we feel that similar organisms from boiled milk which correspond to Loeffler's description should be regarded as identical with his species. We therefore propose to utilize the name *Bacillus albolactus* Migula, (synonym *Bacillus lactis albus* Loeffler) for the organisms isolated from the source studied by Loeffler. This organism is undoubtedly isolated from time to time by other bacteriologists and must exist in a number of laboratories. It was apparently described recently by Neide (1904) in Meyer's laboratory as *Bacillus teres. Bacillus albolactus* is, we believe, the common cause of the clotting and peptonization occasionally seen with boiled milk. It is undoubtedly also a contributing

factor to the changes seen in milk pasteurized at lower temperatures, 60° to 65°C., which subsequently develops a bitter taste.

Bacillus mycoides was identified without difficulty by the classical descriptions and by the work of Chester whose conclusions were based upon a culture which was sent him from our laboratory several years ago.   The felted growths in the depths of agar are very characteristic and are given by but one other species, *Bacillus ramosus-liquefaciens* of Prausnitz.

For a long time we were in doubt as to the identification of the very large microörganisms which are placed in a heterogeneous group and sometimes called *Bacillus megatherium*, sometimes *Bacillus petasites*, and sometimes *Bacillus tumescens*.   The first member of this group was described by De Bary (1884, 1887) whose illustrations are very characteristic.   Many of the cultures identified and sent to us as *Bacillus megatherium* differed radically from De Bary's description, and Chester's conclusions in regard to the ill-defined character of the group seemed to be entirely justified.   These large organisms were very abundant however and soon resolved themselves into two distinct types. One type agreed with De Bary's original description in all essential particulars and this type agreed also with an isolation of *Bacillus megatherium* by Kellermann sent us from the Winslow collection.   Two cultures of *Bacillus tumescens* of Zopf agreed closely with this *Bacillus megatherium* and there seems to be no reason to regard it as a distinct species.   The other type has almost the same morphology and the same cultural reactions as *Bacillus megatherium* but produces an intense yellow pigment. This type corresponds to the organism recently described as *Bacillus petasites* by Gottheil.   All the organisms thus far encountered with the morphology referred to can thus easily be divided into these two main forms.   The *Bacillus graveolens* of Gottheil, not the *Bacillus graveolens* of Bordoni-Uffreduzzi, seems to be merely a strain of *Bacillus megatherium* in which the bacilli have a peculiar property of growing in short spirals.   It has not been encountered in our work.

The *Simplex-cohaerens* group of Chester proved possibly the

most difficult of all to clarify. Two organisms were originally described by Gottheil as distinct species, regarded by Chester however as practically identical. Strains of *Bacillus simplex* and *Bacillus cohaerens* received by us from Kral were quite different morphologically and while it is evident that we lack many of those pronounced cultural and morphological reactions which render species and groups easy to recognize yet we must not therefore place organisms together which are clearly different. On one occasion we found in milk an organism evidently identical with the strain of *Bacillus cohaerens* received from Kral and corresponding to Gottheil's original description. Subsequently this species was found five times by Dr. Laubach in soil. These organisms gave us a fairly clear idea of the species and its differentiation from *Bacillus simplex* whose description we also give here. This latter description while made from a strain isolated by Gottheil, applies also to a species subsequently found in dust by Dr. Laubach. On two occasions we isolated from milk the species described as *Bacillus fusiformis* by Gottheil. Our isolations were identical with Gottheil's in every particular. Finally on one occasion we obtained a strain with properties practically the same as those of the species described by Flügge as No. XII and now known as *Bacillus terminalis* Migula.

The 250 cultures studied were from 68 samples of milk, 12 of raw milk, 12 of milk pasteurized at 60°C., 32 of milk heated to 85°C., and 12 of boiled milk. These cultures may thus be held to represent so many various conditions in the development of the bacteria of milk as to give an accurate idea of the spore-bearing organisms of milk in Baltimore and they probably represent conditions met with elsewhere. By their combined development in heated milk they give rise to the putrid decomposition so frequently observed. As can be seen from their cultural reactions these organisms are in the majority of instances energetic protein-splitters and in practically every case rapidly dissolve the casein in milk either before or after a preliminary coagulation.

After the various types of spore-bearing organisms were established by the study of 250 cultures from the 68 samples

mentioned above another series of milks, also subjected to various treatments, was investigated with the object of testing the preliminary classification adopted. In this second portion of our work the types previously established were abundantly confirmed but no new organisms were isolated. We believe therefore that the organisms first worked out represent the spore-bearing organisms usually present in Baltimore milk. Our original 250 cultures show the various species in the following proportions.

Baltimore Milk

Bacillus cereus Frankland..........................	124
Bacillus subtilis (Ehrenberg) Cohn..................	79
Bacillus albolactus Migula.........................	25
Bacillus vulgatus (Flügge) Trevisan.................	15
(*Bacillus mesentericus vulgatus* Flügge.)	
Bacillus mesentericus (Flügge) Migula...............	2
(*Bacillus mesentericus fuscus* Flügge.)	
Bacillus fusiformis Gottheil.......................	2
Bacillus petasites Gottheil........................	1
Bacillus cohaerens Gottheil........................	1
Bacillus terminalis Migula........................	1

In addition to these the following species were isolated from other sources during the work on milk and made the basis for comparison. We have reason to believe that they may occur in milk, partly from the work of others and partly because they are not infrequent in milk products. We introduce them here for completeness.

Bacillus mycoides Flügge.

Bacillus megatherium De Bary.

Bacillus simplex Gottheil.

Bacillus aterrimus Lehmann & Neumann (*Bacillus mesentericus niger* Lunt).

Bacillus niger Migula (*Bacillus lactis niger* Gorini).

Bacillus globigii Migula. (*Bacillus mesentericus ruber* Globig).

Finally a brief note is added in regard to certain other cul-

tures sent us which we do not regard as entitled to specific rank, namely

Bacillus pumilus Gottheil.

Bacillus graveolens Gottheil.

Bacillus tumescens Zopf.

Bacillus cereus Frankland 1887

This organism was first described by the Franklands in 1887 (Franklands, 1887). It has since been described under a host of names and it is impossible to say how many different species are identical with it. It fs the most widely distributed organism of this group in Baltimore, being found abundantly in milk, soil, dust, water, and in the intestinal contents. It is particularly common as a laboratory contamination. The present description applies to cultures received from the Kral collection, from the American Museum, and from a number of American laboratories and to over a hundred of our own isolations.

Morphology. Regular bacilli with homogeneous protoplasm and rounded ends, in young cultures measuring about 0.75 by 2.25 to 4 microns. Many of the organisms show peculiar refractile bodies of various sizes as the cultures get older, presenting a characteristic appearance. The nature of these bodies is not clear as they do not give reactions for starch or volutin. They can usually be differentiated from the beginning spores. On glucose agar the bacilli are thicker and longer measuring 0.75 to 1 by 3 to 6 microns. Here the entire protoplasm of the organism is converted into the bodies mentioned above. They are globular, highly refractile, and are often as thick as the organism. (Figures 25, 26 and 27.)

Motility. Actively motile in young cultures.

Staining properties. Gram-positive.

Spore-formation. Spores are formed early on both plain and glucose agar, often appearing within 24 hours or even in less time. They may be central in position, excentric or even subterminal but the latter location of the spore is rare. The spores are usually wider than the organisms from which they spring

and thus bulge the rods slightly. The free spores retain their protoplasm at the ends for some time, usually in equal amounts. Often, however, the protoplasm is greater at one end than at the other and the spore then has a characteristic appearance like an enlarged mesentericus spore. The free spores are cylindrical, soon shed their protoplasm and measure 0.5 to 0.75 by 1.125 to 1.5 microns.

Agar slant. Abundant, thick, white mealy growth along the line of inoculation sometimes with arborescent edges. In older cultures the growth is much thicker, yellowish white and may show pellucid areas surrounded by more highly refractive patches.

Agar stab. Little growth along line of inoculation but luxuriant surface growth spreading over entire surface of agar and extending to the walls of the tube.

Agar colonies. Round, raised, dense, highly refractive surface colonies. If slight amount of water of condensation be present the colonies may be amoeboid. Under low power the colonies consist of dense central nuclei with spreading peripheries made up of numerous curling and parallel chains. The colonies are soft and easily lifted from the agar. Deep colonies punctiform, stellate or rhizoid. Under the low power they are fuzzy, irregular and may resemble a chestnut burr.

Litmus glucose agar slant. Thick, yellowish-white growth along the line of inoculation and spreading out over entire surface. The medium is acidified and the growth is sometimes distinctly yellow. Typical cultures rapidly decolorize the litmus and then become alkaline and the agar turns deep blue. Occasionally the cultures are less active alkali-producers and the medium remains permanently acid. Such cultures, however, can usually be stimulated to alkali production by plating and they then give characteristic growths.

Glucose agar colonies. Surface colonies round or bizarre, heaped up, with irregular margins, smaller than plain agar colonies. Under low power granular, with dense central nuclei and irregular margins, showing fine parallel strands. Deep colonies small irregular or round. Under low power they consist of dense central nuclei with fine, irregular or parallel strands in the periphery.

Gelatin stab. Uniform growth along entire line of inoculation with a liquefaction also along entire line. The liquefaction becomes cup-shaped or sacculated with a surface scum. It is rapid and frequently in two days the entire gelatin tube is liquefied.

Gelatin colonies. Loosely filamentous colonies with, dense, central nuclei and spreading irregular margins, often very thin, edges entire. Gelatin liquefied rapidly.

Broth. Very turbid in 24 hours with no scum except occasionally a slight ring growth. In two days a heavy friable scum is produced which is entirely precipitated within a short time. The medium gradually clears while a heavy flocculent precipitate is deposited.

Peptone. Very turbid in 24 hours. Scum appears usually on the second day and is soon precipitated. It is like that produced in broth but is more friable.

Potato. Thick, white, mealy growth in a few days becoming yellowish or brown with a discoloration of the potato. This brownish growth may become very moist and slimy and is occasionally measley but never vermiform. It never assumes an appearance similar to that seen with cultures of *Bacillus subtilis* or *Bacillus vulgatus*.

Litmus milk. With the majority of cultures peptonization begins immediately and progresses rapidly in three zones. Surface zone is amber, middle zone violet, the lowest zone blue. Peptonization continues until the entire milk tube is converted into an amber fluid with a slight sediment. Milk does not coagulate. With some cultures the three-zone appearance does not show but the milk is gradually changed to a muddy gray-colored fluid. Eventually, however, the same clear amber-colored fluid is produced.

Blood serum. Thick, white, dry, smooth growth. No liquefaction.

Fermentation tubes. Glucose. Abundant growth in bowl and arm. Friable scum forms which is soon precipitated. Turbidity gradually disappears and a flocculent precipitate is deposited. Reaction highly acid.

Saccharose. Abundant growth in bowl and arm. Filmy and

friable scum forms which is soon precipitated.   In many cultures the reaction is acid.   Reaction alkaline in the majority of cultures.

Lactose.   Turbidity in bowl with scum formation.   Arm clear.   Reaction alkaline.

Thermal death point.   The spores survive steaming one hour in the Arnold sterilizer and autoclaving at 19 pounds pressure. Killed by 20 pounds pressure.

Bacillus albolactus Migula 1900

This organism was apparently first obtained in pure culture in 1887 by Loeffler who found it in boiled milk which had soured and clotted and who named it *Bacillus lactis-albus*.   It is possibly identical with *Bacillus corrugatus* Migula (1900) (Bacillus No. II Flügge), with *Bacillus bernensis* Lehmann and Neumann, (1901) and with the organism described recently by Neide as *Bacillus teres* which was also obtained from boiled milk which had subsequently soured.   It ·is common in boiled milk in Baltimore and produces the souring, clotting, and subsequent peptonization seen so frequently in this material.

Morphology.   These organisms are identical morphologically with *Bacillus cereus*.   In young cultures 6 to 24 hours old, on plain agar, they have round ends and measure 0.5 to 0.75 by 2.25 to 4 microns.   The protoplasm may be homogeneous or may show globular bodies of various dimensions.   On glucose agar the globular bodies are much more abundant and give the organism a characteristic appearance.   Here the rods measure 0.75 to 1 by 2.5 to 4 microns.   (Figs. 28, 29, and 30.)

Motility.   Actively motile in young cultures.

Staining properties.   Gram-positive.

Spore formation.   Spores are formed readily on plain and on glucose agar.   They are abundant in 24 to 48 hours and have the same appearance as the spores of *Bacillus cereus*.   They are usually central or slightly excentric and a trifle wider than the organisms from which they spring thus bulging the rods somewhat.   The free spores may retain equal or unequal bits of

protoplasm at the ends and thus have a characteristic appearance. They are oval to cylindrical and measure 0.5 to 0.75 by 1.5 to 2.125 microns. The spores are frequently seen in pairs attached by their protoplasmic remnants, and also sometimes in chains.

Agar slant. Luxuriant, thick, white growth with a smooth and glistening surface, spreading over the entire surface of the agar. Some cultures show a delicate transverse wrinkling.

Agar stab. Fine, slightly arborescent growth along line of inoculation. Thick, white, wrinkled surface growth.

Agar colonies. Surface colonies thick, raised, round or bizarre, frequently show'ng dense, central nuclei. Under low power granular with dense, central nuclei and spreading peripheries made up of curved parallel strands. Deep colonies small, round or irregular. Under low power irregular, mossy, with irregular fuzzy margins.

Litmus glucose agar slant. Thick, yellowish-white, moist growth, spreading over the entire agar and wrinkling slightly at the base when the culture is very active. Reaction in medium acid in first few days but gradually alkali is produced and the agar turns dark blue.

Litmus glucose agar colonies. Surface colonies thin, translucent, somewhat smaller than plain agar colonies. Under low power granular with thin peripheries made up of curling parallel strands. Deep colonies round or irregular. Under low power irregular, mossy with irregular, fuzzy margins. Medium first acidified and then made alkaline.

Gelatin colonies. Surface colonies round, spreading concentrically and composed of a central loose mass of filaments denser than the surrounding zone. Deep colonies are composed of spherical masses of loose filaments with irregular, mossy or bristling margins. Rapid iquefaction.

Gelatin stab. Growth along line of puncture with a rapid cup-shaped liquefaction and scum production.

Broth. Turbidity with ring growth in 24 hours and scum formation in 2 to 3 days. Scum quickly precipitated.

Peptone. Turbidity with scum formation on the second day. Scum usually persists.

Potato. Thick, white, moist growth later becoming yellowish brown. Never wrinkled or vermiform, rarely measley. Medium discolored.

Litmus milk. Acid production and coagulation, usually within 24 hours. The coagulum is at first firm but gradually undergoes peptonization, and is usually completely dissolved at the end of three weeks. Odor distinctly faecal and very disagreeable, with a suggestion of indol.

Blood serum. Thick, white growth. No liquefaction.

Fermentation tubes. Glucose. Abundant growth in bowl extending up into closed arm which becomes turbid. Flocculent precipitate forms but usually no scum. Reaction highly acid.

Saccharose. Turbidity in bulb extending up into the closed arm. Flocculent precipitate. No scum. Reaction highly acid.

Lactose. Turbidity in bowl extending up into the closed arm. No precipitate but usually a thick scum is formed. Reaction highly acid.

Thermal death point. Organisms have survived 1 hour in the Arnold sterilizer and autoclaving to 15 pounds pressure. Killed by 16 pounds pressure.

Bacillus subtilis (Ehrenberg) Cohn

Synonyms. *Vibrio subtilis* Ehrenberg 1838; *Bacillus subtilis* Cohn 1872; *Bacillus subtilis* (Ehrenberg) Cohn, Migula 1900.

Considerable difference of opinion exists as to the correct interpretation of the somewhat puzzling literature concerning this organism. In this paper we have followed the views of Chester who has identified a number of organisms isolated in this country as the real *Bacillus subtilis* of Cohn, and who sent one of his isolations to our laboratory several years ago. It is one of the commonest organisms in milk, soil, dust and water. In morphology it is one of the smallest of the aerobic spore-bearing bacteria and is thus easily distinguished from *Bacillus cereus* with which it is most often confused.

Morphology. Small, thin, homogeneous bacilli measuring 0.375 by 1.5 to 2.5 microns in 24 hour agar cultures. Some-

what thicker and longer on glucose agar measuring 0.5 by 1.5 to 4 microns. Does not usually form threads on this medium. (Figures 4 and 5.)

Motility. Sluggishly motile in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed early appearing within 24 hours on plain and glucose agar. They arise in the center or towards one end of the rods and are slightly greater in diameter than the rods, thus causing a distinct bulging. The free spores may retain bits of protoplasm at each end, often unequal in amount, giving the spore a characteristic appearance. Such spores measure about 0.5 by 0.875 microns. The spores rapidly lose their protoplasm, become more oval and measure about 0.5 by 0.75 microns.

Agar slant. Weakly refractive, glassy, membranous growth along line of inoculation, later spreading out over entire surface of agar. The surface is usually dry and hard, but in old cultures it becomes soft and smeary, but is always firmly attached to the agar from which it cannot be scraped off.

Agar stab. Little growth along the line of inoculation but a spreading, dry, membranous growth on the surface of the agar, extending to the wall of the tube.

Agar colonies. Surface colonies weakly refractive, spreading concentrically or in amoeboid fashion from small dense nuclei. Under the low power edges may be complete or finely crenate. If water of condensation be present one or two colonies frequently overgrow the entire plate. Under the low power the colonies are homogeneous and granular or irregular and gyrose. The deep colonies are punctiform and under the lower power lichenlike with irregular margins myceleoid in character. The colonies are usually membranous dry, hard, and glassy, and can be separated from the agar only with great difficulty.

Glucose litmus agar slant. Highly refractive growth verrucose or vesicular, with milky liquid in vesicles, not spreading. Parts of growth show distinct red pigment. Acid is produced in 24 hours, but is replaced by alkali in about ten days, medium turning deep blue.

Litmus glucose agar colonies. Irregular, spreading, bizarre surface colonies, usually more luxuriant than plain agar colonies. Under low power, irregular with entire edges or fuzzy, with myceleoid outgrowths from dense central nuclei. Deep colonies slightly irregular or punctiform. Under low power irregular myceleoid with filamentous edges. Medium first acidified then made alkaline.

Gelatin stab. Slow growth along line of inoculation and rather slow cup-shaped, surface liquefaction with scum production.

Gelatin colonies. Surface colonies round, homogeneous, spreading, thin and granular. Deep colonies yellowish brown, highly refractive. Under low power granular. Colonies may also show dense central nuclei and thin myceleoid filamentous growth extending in every direction through the medium. Gelatin liquefied.

Broth. Single isolated pellicles appear on the surface in 24 hours. In 48 hours these unite to form a thin branching scum, which gradually becomes more dense and tough. Medium grows turbid in first 24 hours, but later clears. Scum is precipitated as a whole in about ten days. This manner of scum formation is characteristic of *Bacillus subtilis.*

Peptone. Turbidity in the first 24 hours and gradual clearing with a flocculent precipitate. Scum on the surface formed in the same manner as on broth, but not so dense or tough. The pellicles often show chains and branching figures. Frequently the scum has a delicate pink color after about five days' growth.

Potato. Growth on potato characteristic. It is luxuriant and warty, having the appearance of many large and small dew drops scattered along the line of inoculation. In 48 hours a pink pigment collects on top of this growth and persists. In older cultures a decided rose-red line in the substance of the potato marks the limit of the growth. In ten days the vesicles dry down and only a reddish-brown dry growth remains on the discolored medium. Later the growth is moist and sticky.

Litmus milk. No change in 24 hours and sometimes none in 48 hours except that the milk becomes more alkaline. In three days the medium begins to clear from the surface, the deeper

parts remaining unchanged. Clearing progresses slowly, the supernatant fluid persisting as a grayish, pinkish or yellowish muddy medium. After a month at room temperature the medium may become very alkaline and turn deep blue-purple. Milk never coagulates.

Blood serum. Vesicular, dew-drop growth with pink color often very marked, in 24 hours. Vesicles dry down eventually leaving a hard wrinkled growth. Medium is not liquefied.

Fermentation tubes. Glucose. Turbidity in bowl and arm. Scum formation like that seen in broth. Highly acid.

Saccharose. Turbidity in bowl and arm with a fragile scum forming from pellicles in about two days. Acid production but not so marked as in glucose.

Lactose. Turbidity in bowl and extending up in the arm to the level of the medium in the bowl. Rest of the arm clear. Dense tough scum. Reaction alkaline.

Thermal death point. Spores survive steaming $1\frac{1}{4}$ hours in the Arnold sterilizer. Survive autoclaving up to and including 19 pounds pressure but usually destroyed by 20 pounds pressure.

Bacillus vulgatus (Flügge) Trevisan.

Synonomy. *Bacillus mesentericus vu'gatus* Flügge 1886; *Bacillus vulgatus* Trevisan 1889; *Bacillus vulgatus* Eisenberg 1891; *Bacillus vulgatus* (Flügge) Migula 1900.

This organism was first described by Flügge in 1886 (Flügge, 1886) and is commonly known as the "potato bacillus." According to Chester it is identical with *Bacillus subtilis*. By the use of glucose agar and blood serum and by the careful observation of the cultural reactions, particularly in broth and on potato the species is easily separated from this organism. It is fairly common in Baltimore but by no means as frequent an isolation as are many of the other spore-bearers.

Morphology. Small homogeneous organisms usually distinctly larger than *Bacillus subtilis*, measuring 0.5 by 2 to 3 microns. Occasionally short forms 1.125 and long forms measuring 4 microns are seen on plain agar. On glucose agar the organisms

are thicker and much longer measuring often nearly 0.75 microns in thickness and 5 microns in length.   (Figures 6 and 7.)

Motility.   Active progressive and rotatory motility in young cultures.

Staining properties.   Gram-positive.

Spore formation.   Spores are formed early appearing in 24 hours on plain and glucose agar.   They arise in the center or towards one end of the rods but do not ordinarily bulge the rods appreciably.   When free they are elongated and flattened and retain tags of protoplasm at each end.   At times the protoplasm at one end is greater in amount then at the other.   Such spores measure about 0.5 by 1.125 microns.   As they lose their protoplasm they become cylindrical measuring about 0.5 by 1 micron.   In general the spores are about the same width as the vegetative rods or only very slightly wider.

Agar slant.   Moist profuse thick growth on agar, easily lifted or brushed from the surface of the medium with the platinum wire.   Growth is usually white or cream white, spreads but little from the line of inoculation and is whitest at the edge where it is heaped up.   When water of condensation washes over the agar many small, round colonies develop apart from the main growth.   In some strains the agar growth is dry and fine wrinkles develop but the growth can always be lifted from the agar with a platinum loop.

Agar stab.   Little growth along line of inoculation but rather dry wrinkled rooty growth spreads over the surface of the agar.

Agar colonies.   Surface colonies round, waxy, highly refractive or spreading and amoeboid with greatest refraction at the edge of the advancing growth, where colonies are thickest. Under low power of the microscope edges entire.   Deep colonies punctiform, round or elliptical.   Under low power they are irregular, brown, slightly granular with entire or fuzzy edges.

Litmus glucose agar slant.   Characteristic appearance.   Luxuriant dry brown and abundantly wrinkled growth develops within 24 to 48 hours.   The medium is acidified.   After a few days the growth usually becomes moist and the wrinkles are obliterated while the medium becomes alkaline and turns deep blue.

Litmus glucose agar colonies. Superficial colonies are thick, highly refractive, waxy, with entire edges or spreading with irregular edges. They soon become dry and wrinkled in the center. Under low power opaque with entire edges. Deep colonies are punctiform, round, oval or irregular with crenated margins. Under low power opaque with irregular margins. Medium acidified at first then turned alkaline.

Gelatin. Stab gives cup-shaped and surface liquefaction with heavy scum production.

Gelatin colonies. Colonies round with highly refractive centers occasionally showing beautiful concentric rings. Under the low power the colonies have a granular appearance. Medium liquefied.

Broth. Turbidity within 24 hours and scum formation usually on second day. Medium gradually clears.

Peptone. Turbidity within 24 hours. Thin fragile scum after the lapse of several days. Medium gradually clears.

Potato. Characteristic appearance. Thick, white, gray or pink folds or wrinkles are formed within 24 to 48 hours often covering entire cut surface of potato. Later these folds dry down to a brown, reticulate mass. Potato usually discolored.

Litmus milk. Slight clearing of the milk just beneath the cream layer usually appears in 24 hours. This reaction rapidly intensifies with the production of a clear fluid colored deep Chinese-blue or purple. No acid production or coagulation. As the milk gets older complete peptonization occurs with the formation of a clear amber-colored fluid.

Blood serum. Thin dry abundant growth usually smooth, but sometimes wrinkled and pink. Growth later becomes moist and gives a suggestion of liquefaction.

Fermentation tubes. Glucose. Luxuriant growth in bulb gradually extending into the closed arm. An abundant scum is formed which may be quite wrinkled. Reaction acid.

Saccharose. Growth luxuriant in bowl but scanty in arm. Very thin scum may be formed after several days, but this may be lacking. Reaction varies from slight to marked acidity.

Lactose. Abundant growth in bowl with late scum production. No growth in closed arm. Reaction alkaline

Thermal death point. Organisms survived heating in broth in the Arnold sterilizer for one hour. Survived autoclaving up to and including 19 pounds pressure, but were destroyed by 20 pounds pressure.

Bacillus mesentericus (Flügge) Migula 1900

This organism was first described by Flügge in 1886 (Flügge, 1886) as a species distinct from *Bacillus mesentericus-vulgatus* and named *Bacillus mesentericus-fuscus*. We have isolated a number of organisms which correspond to the description given by Flügge and also by Chester. It is one of the less common of the aerobic spore-bearing bacteria but occurs in milk, soil, dust and water.

Morphology. Organisms about the same in morphology as *Bacillus mesentericus-vulgatus*. On agar cultures in 24 hours they are homogeneous rods measuring 0.5 by 1.5 to 3 microns. Sometimes shorter forms predominate in the cultures, a little over a micron in length. On glucose agar they are thicker and longer measuring 0.75 by 2 to 5 microns, with many long forms measuring 6 to 8 microns in length. (Figures 8 and 9.)

Motility. Active motility, progressive and rotatory, in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form in 24 hours on plain and on glucose agar. By the end of 48 hours they are very abundant. They appear in the center or towards one end of the rods and do not bulge the organism appreciably. The free spores are cylindrical and may retain equal bits of protoplasm at each end or this protoplasm may be unequal in amount giving a characteristic appearance to the spore. They measure about 0.5 by 1.125 microns. They rapidly lose their protoplasm and become slightly more oval, measuring 0.5 by 0.75 microns.

Agar slant. Soft white or cream-white growth somewhat translucent when old, spreading but little from the line of inoculation except in the presence of water of condensation. Easily lifted from the agar. Edges of growth irregular or serrate. Growth does not become dry or wrinkled.

Agar stab. Little growth along line of puncture, luxuriant growth on surface.

Agar Colonies. Superficial colonies round highly refractive with entire edges, or spreading and amoeboid. Under low power opaque with crenated edges. Deep colonies round and regular. Under low power slightly granular with crenated margins.

Litmus glucose agar slant. Thick, abundant, white or cream white to yellow growth spreading along the line of inoculation. Medium first turns acid but as growth becomes older it again becomes deep blue.

Litmus glucose agar colonies. Superficial colonies round, highly refractive, with entire edges or spreading and amoeboid with densest part of the growth along the advancing edge. Under low power of the microscope edges crenated. Deep colonies round or oval and under low power slightly granular with crenated margins. Medium first acidified and then made alkaline.

Gelatin stab. Cup-shaped or surface liquefaction and scum production.

Gelatin colonies. Colonies dense with liquefaction centers and granular ring at the edges of a cup-shaped liquefaction.

Broth. Turbidity and a rather fragile scum appears late. Medium then clears.

Peptone. Turbidity with small patches of surface growth. Medium soon clears.

Potato. Growth abundant, moist, brown with finely wrinkled or lichen-like appearance in the majority of instances. At times the fine wrinkling is lacking and only a thick, moist, brown, mealy growth is produced.

Litmus milk. Slow peptonization with the production of a lilac color turning to amber. In a few weeks digestion is complete and only a white sediment is left behind. No acidification No coagulation.

Blood serum. Thin, white, dry, at times finely wrinkled growth which later becomes yellowish and moist. Suggestion of liquefaction, but this is never complete.

Fermentation tubes. Glucose. Turbidity and scum in bulb and turbidity in closed arm. Reaction acid.

Saccharose.   Turbidity in open bulb and usually no scum. Turbidity in closed arm.   Reaction acid.

Lactose.   Turbidity in open bulb.   No scum.   Arm clear. Reaction alkaline.

Thermal death point.   Spores survive one hour's heating in Arnold sterilizer and autoclaving at 19 pounds pressure.   Killed by 20 pounds pressure.

Bacillus globigii Migula.

This organism was originally described by Globig (1888) as *Bacillus mesentericus-ruber.*   A culture was obtained from Kral's Laboratory in Vienna which has the same cultural reactions as those given by Globig.

Morphology.   Homogeneous bacilli measuring 0.5 by 2 to 3 microns in 24 hours agar cultures.   On glucose agar the organisms are longer and slightly thicker often growing out into long chains but short forms are also frequently seen   (Figure 14).

Spore formation.   Spores are formed very sparsely and at a late period in the present culture.   They are usually seen only in 16 to 18 days growth and are then characteristic mesentericus spores.

Motility.   Actively motile in 24 hour cultures.

Staining properties.   Gram-negative.

Agar slant.   Thin, spreading, glassy, soft, yellowish-white growth along line of inoculation.

Agar stab.   Slight uniform growth along line of puncture with spreading amoeboid surface growth.

Agar colonies.   Dense, soft, white amoeboid colonies similar to those of *Bacillus mesentericus.*

Litmus glucose agar slant.   Thick, narrow, white growth along line of inoculation.   The medium shows an acid reaction.

Litmus glucose agar colonies.   Thick, round, raised, soft colonies later turning yellowish and rarely pinkish.   Medium acidified.

Gelatin stab.   Growth along line of inoculation and slight surface growth with liquefaction of the gelatin.

Gelatin colonies.   Surface colonies round, granular, punctiform with slow liquefaction.   Some of the larger colonies are spreading and have a glassy surface.   Deep colonies punctiform, spherical with dense centers.

Broth.   Slight turbidity with no surface growth but a flocculent precipitate.   Medium is eventually turned dark yellow.

Peptone.   Slight turbidity with no surface growth and no precipitate.

Potato.   Yellow, moist growth becoming a reddish brown. Medium is discolored.

Milk.   No change in 48 hours.   In twenty days the medium shows an acid reaction with the precipitation of a white sediment.

Blood serum.   Thick, transparent spreading growth with irregular edges.

Fermentation tubes.   Glucose.   Turbidity in open bulb.   No scum.   No growth in closed arm.   Reaction acid.

Saccharose.   Turbidity.   No scum.   No growth in closed arm.   Reaction alkaline.

Lactose.   Turbidity.   No scum.   No growth in closed arm. Reaction alkaline.

Thermal death point.   Spores withstood one hour's sterilizing in the Arnold sterilizer, survived autoclaving at 15 pounds pressure but were killed by 16 pounds pressure.

Bacillus aterrimus Lehmann & Neumann.

This organism was originally described by Biel (1896) and named by Lunt (1896) *Bacillus mesentericus-niger.*   It is not uncommon in milk, soil, and the intestinal contents of man.

Morphology.   Bacilli similar to *Bacillus vulgatus* in morphology.   On plain agar they are homogeneous with blunt ends and measure about 0.5 by 2 to 3 microns in dimensions.   On glucose agar they are thicker and longer measuring 0.75 by 2 to 4 microns but at the same time shorter forms are frequent measuring 0.75 by 1.5 microns.   (Figures 10 and 11.)

Spore formation.   Spores are formed early appearing in 24 hours on plain agar.   They form in the center or towards one

end of the rods which do not swell appreciably. When free they may retain spurs of protoplasm at each end unequal in quantity and measure about 0.5 by 1.5 microns. The spores rapidly lose their rims of protoplasm and are then oval to cylindrical measuring 0.5 by 0.75 microns.

Motility. Actively motile in 24 hour cultures.

Staining properties. Gram-positive.

Cultural reactions. This organism is identical with *Bacillus vulgatus* in all its cultural reactions except that it imparts a distinct color to the various media. This color varies from a steel grey to a brown or black and is best seen on solid media. It is very pronounced on potato where the characteristic folds of the "*vulgatus*" are converted to thick black wrinkling bands.

Thermal death point. The spores resist an hour's steaming in the Arnold sterilizer and 15 pounds pressure in the autoclave. They are destroyed by 16 pounds pressure.

Bacillus niger Migula 1900

This organism was first described by Gorini (1894) in 1894 as *Bacillus lactis-niger* and is closely related to the preceding organism. A culture obtained from Kral's Laboratory shows the following reactions.

Morphology. Bacilli with homogeneous protoplasm and blunt or rounded ends measuring 0.375 to 0.75 by 1.5 to 3 microns in 24 hour agar cultures. No change in morphology on glucose agar. (Figures 12 and 13.)

Spore formation. Spores are formed in 24 hours on plain agar and in 48 hours on glucose agar. They appear in the center or towards one end of the rods and are oval or cylindrical in shape. The free spores may retain protoplasm at both ends and are typical of the "mesentericus" group. They measure 0.75 to 1 by 1.125 to 1.25 microns in dimensions.

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Cultural reactions. This species has the general cultural reactions of *Bacillus mesentericus*. It grows on agar as a rather

thick moist mass with a silvery sheen which shows black areas at the edges and in old cultures imparts a black tone to the agar. It liquefies gelatin rapidly, produces a faint acidity in milk which it first coagulates and then slowly digests. On glucose agar it tends to wrinkle slightly. It produces a faint acid in glucose, saccharose and lactose fermentation tubes. On potato it grows as a raised brown mass and it also produces a brownish growth on blood serum.

Thermal death point. The spores withstand boiling one hour in the Arnold sterilizer and a pressure of 20 pounds in the autoclave. They are destroyed by a pressure of 22 pounds.

Bacillus pumilus Gottheil 1901

An organism described by Gottheil (1901) in 1901 as *Bacillus pumilus* is regarded by Chester as identical with *Bacillus mesentericus*. A culture of *Bacillus pumilus* received from Kral's collection in Vienna has all the morphological, tinctorial, developmental and cultural reactions of this species.

Bacillus mycoides Flügge 1886

This organism was first described by Flügge (1886) in 1886 and has since then been given other names by various authors. It is not the same as *Bacillus ramosus-liquefaciens* of Prausnitz which is a distinct species. *Bacillus mycoides* is quite common in Baltimore and is present in milk, water, soil, and dust.

Morphology. In young cultures 6 to 8 hours old on plain agar the organisms are homogeneous with square ends and measure usually a little more than 0.5 micron in width by 3 to 6 microns in length. They are distinctly thinner and longer than *Bacillus cereus*. As the organisms mature the protoplasm appears more granular and a characteristic arrangement in short and long chains is seen. They then resemble the anthrax bacillus. On glucose agar the bacilli are thicker, 0.75 to 1 micron, and usually about the same length. On this medium the protoplasm is converted into globular bodies which do not take the stain and which are similar to those seen in *Bacillus cereus*. In certain

instances the organisms seem to be made up of a network of fine strands in which the globular bodies hang suspended. Often the chains are curled or curved upon themselves. Old cultures show an abundance of swollen involution forms, which seem to have a skein-like arrangement. (Figures 22, 23 and 24.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form early, appearing first as small refractile bodies in the centers or towards one end of the organisms usually at the end of 24 to 48 hours. Gradually the organisms swell and the spores at the same time increase in size and at this stage a long chain of organisms each containing a spore may often be seen. The protoplasm soon disintegrates leaving a rim about the spore which is round or oval or slightly rectangular. Such spores measure 0.75 to 1 by 1.125 microns. Other spores are more definitely elongated and may measure 0.75 to 1 by 1 to 2 microns. The spores often remain attached to each other in short or long chains. The spores vary more in size than do others of this group and may show small forms 0.375 to 0.5 by 0.5 to 1 and large forms measuring 1.125 by 2 lying side by side.

Slant agar. Filamentous rhizoid growth spreading from the line of inoculation and extending into the agar. This growth is at first glassy and glistening, but later grows dull and soft. Appearance on agar characteristic.

Agar stab. Faint arborescent growth along line of inoculation with a surface development in concentric zones.

Agar colonies. Surface colonies spread from dark dense nuclei and show dense, rhizoid peripheries extending into the agar on all sides. Under low power the periphery of the colony is found to be composed of parallel strands of growth. Deep colonies have almost the same appearance and always exhibit the spreading peripheral myceleoid outgrowths.

Litmus glucose agar. Thin membranous myceleoid growth later becoming branched and reticulate. Growth at first moist and white, later becoming pale yellow. Medium first acidified and then turned deep blue.

Litmus glucose agar colonies. Surface colonies thin, round or irregular. Under low power found to consist of masses of matted filaments with usually dense central nuclei, from which single or parallel strands extend into the agar in every direction for long distances. Deep colonies exhibit the same small, punctiform and matted myceleoid growth, under lower power. Medium first acidified and then made alkaline.

Gelatin stab. Filamentous growth along line of inoculation with surface liquefaction.

Gelatin colonies. Colonies consist of dense central nuclei with matted edges from which long strands emerge. The colonies present a peculiar appearance like a chestnut burr.

Broth. No turbidity but a firm scum forms which is soon precipitated.

Peptone. No turbidity, but a flocculent suspension and a firm scum which is soon precipitated.

Potato. Mealy white, later becoming brownish.

Litmus milk. Slow peptonization to an amber-colored fluid. No acidification. No coagulation.

Blood serum. Dry, myceleoid interlacing luxuriant growth. No liquefaction.

Fermentation tubes. Glucose. Flocculent growth in bowl and arm. Scum forms and is soon precipitated. Reaction acid.

Saccharose. Flocculent in bowl and in arm. Scum is formed and precipitated. Some cultures produce moderate acidity. Others produce no acid.

Lactose. Growth in open bulb with a slight extension into arm. Scum formed and soon precipitated. Reaction alkaline.

Thermal death point. Spores survived one hour in the Arnold sterilizer and 15 pounds pressure in the autoclave. Destroyed by 16 pounds pressure.

Bacillus megatherium De Bary

This organism was originally found and named by De Bary (1884, 1887) and has since beeen described under a variety of names by a number of authors. It is one of the most common of

the spore-bearing bacteria and has been found in dust, soil, milk, water, and as a laboratory contamination. The present description applies to cultures obtained from the Kral collection, and from the American Museum, and to over a hundred of our own isolations.

Morphology. These are the largest of the spore-bearing organisms. On plain agar in young cultures, from 8 to 24 hours old, they are long and thick with homogeneous or slightly granular protoplasm, measuring 0.75 to 1.25 by 3 to 9 microns. On glucose agar they are even thicker measuring 1.25 to 1.5 in width. On both media long forms occur but especially on glucose agar. These may measure 30 to 45 microns in length and may show homogeneous protoplasm without evident segmentation. The protoplasm of the organism is at first homogeneous, but by the end of 24 to 48 hours it is converted into a mass of globular bodies resistant to the stains. These globular bodies are clear, highly refractile, bulge the organism somewhat, and are quite numerous six to eight appearing in each rod. They thus give the organism a peculiar and characteristic appearance. They show most markedly on glucose agar but are also present on plain agar where they can best be demonstrated by decolorizing an over-stained preparation. Their nature is not clear as they do not take any special bacterial stains. Shadow or transparent forms appear in *Bacillus megatherium* early, both on plain and glucose agar. These measure 1.125 to 1.5 by 4 to 10 microns, take the stain very faintly and show peculiar bodies of agglomerated protoplasm at the sides or sometimes at the ends. These transparent forms are often thicker and longer and may even measure 2 by 40 to 45 microns. Occasionally they are distinctly· oval with rounded ends measuring about 1.5 by 4 microns and show a small bunch of cytoplasm at the side. When these forms are in chains they are exactly like the original pictures of De Bary. (Figures 31, 32, 33, 34, 35, 36, 37).

Motility. Active progressive and rotatory motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed abundantly on plain

agar in 24 hours and on glucose agar in 48 hours. They appear in the center or slightly towards one end of the rods and are usually of the same diameter but may be slightly thicker. Sometimes two spores seem to arise in one rod but these may possibly be in a rod just prior to division. In general each rod has a single spore. The spores occasionally lie obliquely in the rods. Frequently two spores are at opposite ends of rods lying in juxtaposition and these may remain attached in chains and present a characteristic appearance. The free spores retain protoplasm at the ends for some time. When this is unequal in amount the spore has somewhat the shape of a tennis racket and handle. The free spores are oval to cylindrical and measure 0.75 to 1.125 by 1.5 to 2 microns. They are often flattened on one side having an appearance described as kidney shaped or reniform. The spores show great variations in size more so than do those of the other members of this group.

Slant agar. Thick, raised, soft, white or cream-colored growth which shows a pink tinge by reflected light, with many small, minute pellucid areas. As the cultures get older the growth becomes pale yellow.

Agar stab. Slight growth along line of inoculation, heaped up and spreading slightly on surface. Later surface growth becomes slightly pinkish.

Agar colonies. Surface colonies round, thick, white or cream-colored, highly refractive, turning pale yellow or yellowish-brown in old cultures. Under low power slightly granular, brownish yellow, with entire margins. Deep colonies punctiform. Under low power round or irregular with entire edges, brown and granular.

Litmus glucose agar slant. Thick, luxuriant growth along line of inoculation, at first white and then pale yellow or cream-colored. Medium is first acidified but later becomes alkaline and changes from a dark blue to a smoky brown while the growth becomes a dark gray or smoky brown.

Litmus glucose agar colonies. Large, round, raised surface colonies, cream colored to pale yellow, with heaped up central nuclei. Under low power dark, slightly granular with entire

edges. Deep colonies punctiform. Under low power dark, irregular, bizarre, with entire edges. Medium is acidified and then made alkaline.

Gelatin stab. Funnel-shaped liquefaction. No scum.

Gelatin colonies. Round colonies with concentric zones of growth. Under low power cloudy central nuclei with filamentous peripheries.

Broth. Turbidity but no scum.

Peptone. Turbidity but no scum.

Potato. Thick, white, mealy growth later becoming pale or cream yellow.

Litmus milk. No change within 24 hours then a gradual peptonization with the production of a port-wine colored fluid. No acidification. No coagulation.

Blood serum. Thick, white, moist, heavy growth cream white to yellow in color. No liquefaction.

Fermentation tubes. Glucose. Turbidity in open bulb. No scum. No growth in closed arm. Acid production feeble.

Saccharose. Turbidity in open bulb. No scum. No growth in closed arm. Acid production feeble.

Lactose. Slight turbidity in open bulb. No scum. No growth in closed arm. Reaction alkaline.

Thermal death point. Spores withstood 1 hour steaming in the Arnold sterilizer and 18 pounds pressure in the autoclave. Killed by 19 pounds pressure.

Bacillus petasites Gottheil 1901

This organism was described originally by Gottheil (1901) in 1901. Its chief point of differentiation from *Bacillus megatherium* is that it produces a distinct yellow pigment on artificial media, particularly on plain agar and on potato. It is extremely common, having been found in dust, soil, water, milk, and various milk products. The present description applies to a culture from the Kral collection and to over a hundred of our own isolations.

Morphology. The organisms do not differ appreciably in morphology from *Bacillus megatherium*. They are homogeneous

or slightly granular rods measuring 0.75 to 1.5 by 3 to 6 microns on plain agar in young cultures (8 to 24 hours), and 1 to 1.75 by 3 to 6 on glucose agar. Long forms measuring 12 to 25 microns are seen on plain and on glucose agar. Shadow forms with faintly staining protoplasm, like those seen in *Bacillus megatherium* are common as well as the peculiar refractile globular bodies. (Figures 38, 39, 40, 41 and 42.)

Motility. Active progressive and rotatory motility in young cultures.

Staining reactions. Gram-positive.

Spore formation. Spores are formed abundantly in 24 hours on plain and on glucose agar. The spores are oval to rectangular, of about the same width as the rods from which they spring and frequently form long chains. The free spores may retain tags of protoplasm but soon lose them and then show great variations in size and shape. They may be nearly round, oval, rectangular and reniform and measure 0.75 to 1 by 1.5 to 2 microns.

Agar slant. Thick, moist, abundant mealy growth at first slightly pinkish by reflected light, then becoming bright lemon yellow. Agar slightly discolored.

Agar stab. Slight growth along line of inoculation with heaped up yellowish growth on surface.

Agar colonies. Surface colonies thick, white or yellow, highly refractive. Under low power dark, granular with entire or myceleoid edges. Deep colonies punctiform. Under low power irregular, with irregular edges showing myceleoid, rooty or fuzzy edges.

Litmus glucose agar. Luxuriant, thick, heaped-up growth at first yellow then assuming an orange and then a dark-brown color. Reaction of medium first acid then alkaline. It eventually becomes smoky-brown.

Litmus glucose agar colonies. Surface colonies round, regular and thick or thin and spreading. Under low power granular with entire edges. Deep colonies punctiform. Under low power granular, irregular, with fuzzy edges. Reaction of medium acid at first then alkaline.

Gelatin stab. Growth along line of inoculation with funnel-shaped surface liquefaction. No scum formation.

Broth. Turbidity. No scum. Medium eventually becomes yellow.

Peptone. Turbidity. No scum.

Potato. Thick, mealy, bright yellow growth gradually becoming dark yellow.

Litmus milk. No change in 24 hours then a gradual peptonization with the production of a port-wine-colored fluid.

Blood serum. Thick, dry, yellowish, moist growth becoming pale to bright yellow. No liquefaction.

Fermentation tubes. Glucose. Slight turbidity in bulb. No scum. No growth in closed arm. Feeble acid production.

Saccharose. Slight turbidity in bulb. No scum. No growth in closed arm. Faint acid production.

Lactose. Slight turbidity in bulb. No scum. No growth in closed arm. Reaction alkaline.

Thermal death point. Spores survived steaming in Arnold sterilizer 30 minutes, but were killed by 1 hour exposure. Withstood 19 pounds pressure in autoclave but were killed by 20 pounds pressure.

Bacillus tumescens Zopf 1885

This organism was described by Zopf in 1885 (Zopf, 1885). A culture received from the Kral collection and another received from the American Museum agree in their morphological, developmental, tinctorial and cultural features all of which are identical with those of *Bacillus megatherium*. (Figures 45, 46 and 47.)

Bacillus graveolens Gottheil 1901

This organism was described in 1901 by Gottheil (1901) as a new species. A culture from the Kral collection in Vienna has all the cultural reactions of *Bacillus megatherium*. Morphologically it is about the same size, forms spores in the same way, is Gram-positive, produces globular bodies on plain and glucose agar and undergoes involution with the formation of shadow or

washed-out forms. It shows however a distinct tendency to produce curved or spiral forms. On the basis of this one characteristic it is hardly justifiable to make it a distinct species. It should be noted that this use of the term *"graveolens"* is probably incorrect since a *Bacterium graveolens* was described by Bordoni Uffreduzzi (1886) in 1886. This was a small non-sporulating bacillus producing a green pigment. (Figures 43 and 44.)

Bacillus cohaerens Gottheil 1901.

This organism was described by Gottheil (1901) in 1901 but according to Chester it is identical with *Bacillus simplex*. The culture of *Bacillus cohaerens* received from the Kral collection is distinct from *Bacillus simplex* and is represented by four organisms isolated in Baltimore, one from milk and three from soil. The present description applies to all five strains.

Morphology. Small, rather uniform homogeneous organisms with rounded ends, measuring 0.375 to 0.5625 by 0.75 to 2.25 microns in 24 hour cultures on plain agar. On glucose agar the bacilli are thicker and longer measuring 0.5625 to 0.75 by 2 to 5 microns. On both media shadow forms appear early often in 24 hours. These are made up of faintly-staining protoplasm with deeply-staining particles in various positions, at the ends, towards the center, or at the periphery. (Figures 15, 16, and 17.)

Motility. Actively motile in 24 hour cultures.

Staining properties. Gram-positive.

Spore formation. Spores were formed slowly and sparsely in the Kral culture and in one of ours. They appeared in about 10 days, were oval or elliptical, arose in the centers of the rods which were slightly bulged on sporulation. The free spores were very delicate and stained with difficulty. They measured about 0.5625 by 0.75 microns. In a more recent isolation of our own the spores appeared in 48 hours, were central or excentric, bulged the rods and later retained distinct rims of protoplasm, measuring 0.75 by 1.5 to 1.5 microns. Later the spores lost their protoplasm, became more oval and measured 0.5 to 0.5625 by 0.9375 to 1.25 microns. Rarely the spores retained unequal

bits of protoplasm at the ends and then they resembled the mesentericus spores slightly.

Agar slant. Thin, soft spreading, whitish growth later becoming yellow. Easily scraped off the agar.

Agar stab. Faint growth along line of inoculation and spreading on the surface, thick and whitish in old cultures.

Agar colonies. Surface colonies round or bizarre, thick, white. Under low power granular with dense central nuclei. Edges entire. Deep colonies punctiform. Under low power irregular, with entire edges.

Litmus glucose agar. Thick, soft, whitish growth along line of inoculation becoming yellowish and irregularly heaped up. Medium quite markedly acidified.

Litmus glucose agar colonies. Surface colonies round or irregular, thick, whitish. Under low power granular and frequently show dense central nuclei with thin peripheries showing regular edges. Deep colonies punctiform. Under low power irregular with irregular edges. Reaction of medium acid.

Gelatin stab. Faint growth along line of inoculation with surface liquefaction and scum production.

Gelatin colonies. Thin, circular colonies, under low power granular.

Broth. Turbidity at first, then the medium clears and a dense surface growth appears which shows many clear, globular masses like globules of fat floating on the surface.

Peptone. Turbidity with a faint fragile scum.

Potato. Thin, spreading, moist, yellow growth.

Litmus milk. Slow decolorization of the litmus with peptonization and the production of an amber-colored fluid.

Blood serum. Thin, whitish growth. No liquefaction. May appear finely wrinkled.

Fermentation tubes. Glucose. Turbidity in bowl with surface growth and flocculent precipitate. Arm clear. Reaction acid at the end of 2 to 3 days.

Saccharose. Turbidity in bowl with slight surface growth. Arm clear. Acidity at the end of 2 to 3 days.

Lactose. Turbidity in bowl with very slight surface growth. Arm clear. Reaction alkaline.

Thermal death point. In one isolation the spores survived one hour steaming in the Arnold sterilizer; and withstood 18 pounds pressure in the autoclave but were killed by 19 pounds pressure. In another isolation from soil the spores survived 14 pounds pressure in the autoclave but were killed by 16 pounds. They survived one hour steaming in the Arnold.

Bacillus simplex Gottheil 1901

This organism was described by Gottheil (1901) in 1901 as a distinct species. According to Chester it is the same as *Bacillus cohaerens* of Gottheil. Cultures of both organisms have been received from Kral's Laboratory in Vienna and can easily be differentiated. The present description applies to the Kral culture and to an organism obtained from soil by Dr. Laubach. The species is evidently one of the rare spore-bearing organisms.

Morphology. In the Kral culture the organisms are large homogeneous rods with rounded ends, measuring usually 0.5625 to 0.75 by 3 to 4.5 microns. At times much thicker forms are seen approximating 1.125 micron in thickness while longer forms 6 microns in length are not uncommon. The organisms often grow out into long threads or filaments 10 to 12 microns in length, especially on glucose agar. Even in young cultures the homogeneous rods lose their protoplasm and are converted into peculiar shadow forms. These are made up of a very faintly staining protoplasm in which denser aggregations of cytoplasm appear. Such forms measure 1.125 to 1.25 by 12 to 15 microns in dimensions. On glucose agar the organisms have the same morphology but may show an abundance of shadow forms. Involution and shadow forms are very abundant in old cultures. In our own isolation the organisms, while somewhat smaller, did not differ appreciably in morphology, measuring 0.5 to 0.5625 by 1.5 to 2.5 microns but also showing both the thicker and longer forms seen in the Kral culture and the characteristic shadow and involution forms. Long forms were also very common on glucose agar. (Figures 18, 19, 20, and 21.)

Motility. Actively motile in young cultures.

Staining properties. Gram-positive.

Spore formation.   In the Kral culture the spores were at first formed very slowly appearing only after the lapse of 15 to 16 days.   Subsequently after repeated transfers, spore formation became more active and spores were often formed in 24 hours. They appeared in the centers or towards one end of the rods, were no thicker than the rods from which they sprung, and were cylindrical or almost rectangular in shape.   They retained rather thick walls of protoplasm for some time and measured 0.5625 by 1.125 to 1.25 microns.   In our own isolation the spores were formed in 48 to 72 hours in the same way as in the Kral culture but were a trifle smaller measuring 1.375 to 0.5 by 0.75 to 1 micron.

Agar slant.   Thin, translucent, slightly yellowish gelatinous growth, gradually becoming denser and developing occasionally a dry slightly wrinkled surface.   Single accessory colonies not uncommon at the edges of the main growth.

Agar stab.   Slight uniform growth along line of puncture with a thick circular surface growth.

Agar colonies.   Surface colonies thin, translucent, amoeboid developing from pin-point centers.   Under low power granular. Deep colonies round or oval, regular, granular, with clean or rarely irregularly fuzzy edges.

Litmus glucose agar.   Thick, abundant yellowish-white, heaped up growth with serrated margins.   Medium faintly acidified in old cultures.

Litmus glucose agar colonies.   Superficial colonies thin, smooth, white and soft.   Under low power granular, edges irregular but entire.   Deep colonies punctiform.   Under low power irregular with irregular rarely fuzzy margins.   A trace of acid usually produced.

Gelatin stab.   Faint growth along line of inoculation with cup-shaped surface liquefaction.

Gelatin colonies.   Round, thick, whitish colonies with concentric rings and sharply defined edges.   Medium liquefied.

Broth   Faint turbidity, slight sediment, no scum but rarely a faint ring growth along side of tube.

Litmus milk.   Gradual clearing with production of straw-colored fluid in the Kral culture.   In our own isolation a gradual

clearing to a port-wine fluid. No coagulation. Later straw-colored.

Peptone. Faint turbidity and sediment with rarely a slight ring growth.

Potato. Thick, moist, abundant, gelatinous, yellowish-brown growth.

Blood serum. Thin, spreading, whitish growth. No lique-faction.

Fermentation tubes. Glucose. Turbidity in open bulb. No scum, arm clear. Reaction neutral or slightly acid.

Saccharose. Faint turbidity in bulb. No scum. Arm clear. Reaction alkaline.

Lactose. Faint turbidity in bowl. No scum. No growth in closed arm. Reaction alkaline.

Thermal death point. In the Kral culture the spore survived steaming in the Arnold sterilizer for 15 minutes. They withstood a pressure of 15 pounds in the autoclave but were destroyed by 16 pounds pressure. In our own isolation the spores survived 10 pounds in the autoclave but were killed by 12 pounds pressure. They survived 15 minutes steaming in the Arnold sterilizer but were killed by 30 minutes steaming.

Bacillus fusiformis[2] Gottheil 1901

This organism was first described by Gottheil (1901) in 1901. A transfer from Gottheil's original was obtained from Kral's Laboratory in Vienna. Fourteen organisms corresponding closely to Gottheil's isolation were obtained in Baltimore, two from milk, four from dust, two from water, five from soil and one from contaminated hirudin. The present description applies to all of them.

[2] *Bacillus fusiformis* has practically the same morphology and the same cultural reactions as the organism described in 1909 by Jordan and Harris as the cause of milksickness and named by them *Bacillus lactimorbi* (Journal of Infectious Diseases, Vol. 6, No. 4, September 20, 1909, p. 401). A culture of *Bacillus lactimorbi* received from the Winslow collection in New York does not differ appreciably in its reactions from the strains of *Bacillus fusiformis* in our laboratory. Without a thorough study of pathogenicity, however, it is impossible to state whether the organisms found by us are identical with *Bacillus lactimorbi* or not.

Morphology. Thick stubbed homogeneous organisms with round or pointed ends usually appearing as single cells or in twos. No chain formation. On 24 hour plain agar cultures they measure 0.5 to 0.75 by 1.5 to 2 microns. Organisms not increased in size on glucose agar and protoplasm remains homogeneous. Sometimes long forms 6 to 8 microns appear in old cultures. (Figures 48, 49, 50 and 51.)

Motility. Active progressive and rotatory motility in 24 agar cultures.

Staining properties. Gram-negative.

Spore formation. Spores form early appearing in 24 hours on both plain and glucose agar. They are round, greater in diameter than the organisms from which they spring, and are usually located at the ends of the rods in a terminal or subterminal position. They thus give a clavate or club-shaped appearance to the rods which resemble somewhat the tetanus bacillus. The spores may also be central and the rods thus become fusiform in shape. The free spores may retain spurs of protoplasm assuming a peculiar diamond shape or may appear naked. They vary in diameter from 0.5 to 1 micron and are occasionally swollen equaling 1.5 microns in thickness.

Agar slant. Thick white rather dry growth in 24 hours, becoming distinctly yellow or cream-colored in old cultures. Easily scraped from medium.

Agar stab. Faint line growth and non-spreading surface growth.

Agar colonies. Superficial colonies may be round, regular thick and opaque, or thin and spreading. Under low power they show dark central nuclei and thinner margins with clean-cut edges. Older cultures thick and heaped up. Deep colonies small and fine, under low power dark, opaque, round or irregular.

Glucose agar. Thick dry growth with heaped-up edges becoming thicker and granular in old cultures. Reaction alkaline.

Glucose agar colonies. Superficial colonies thick, irregular spreading and heaped up. Under low power granular with irregular fuzzy margins. Deep colonies opaque under low power showing irregular fuzzy edges. Older colonies thicker and more bizarre-shaped. Reaction alkaline.

Gelatin stab.   Growth along line of inoculation with cup-shaped or funnel-shaped liquefaction.   Dense turbidity in the liquefied gelatin with a thick scum.   Gelatin may be faint pink in color.

Gelatin colonies.   Small fine colonies round and regular or irregular and spreading.   Under low power they show fine hairy outgrowths.   Gelatin slowly liquefied.

Broth.   Turbidity and fine sediment.   No scum.

Peptone.   Turbidity and fine sediment.   No scum.

Potato.   Faint yellow growth becoming yellowish brown in old cultures.

Litmus milk.   Gradual reduction of the litmus and slow but complete digestion of the proteins.   No coagulation.

Blood serum.   Non-spreading cream yellow growth becoming yellowish brown in old cultures.   No liquefaction.

Fermentation tubes.   Glucose.   Turbidity in bowl.   Arm clear. No scum.   Reaction alkaline.

Saccharose.   Reactions the same.

Lactose.   Reactions the same.

Thermal death point.   Spores destroyed by steaming 15 minutes in the Arnold sterilizer.   They survive $7\frac{1}{2}$ pounds in the autoclave but are destroyed by 10 pounds pressure.

Bacillus terminalis Migula 1900

This organism was first obtained by Flügge (1894) in 1894 and called by him, No. XII.   It was subsequently correctly named *Bacillus terminalis* by Migula and still later named *Bacillus lacteus* by Chester (1901).   On two occasions we have isolated organisms which have the same morphology and method of spore-formation as *Bacillus terminalis* but differ slightly in cultural reactions.   It does not seem wise to make a new species since our strains may represent merely attenuated varieties of Flügge's organism.   The following description is taken from our own isolations and the points of differentiation between them and Flügge's original isolation are indicated.

Morphology.   Long thin bacilli with slightly granular protoplasm measuring 0.375 by 2.25 to 4 microns in 24 hour agar cul-

tures. On glucose agar the organisms retain the same diameter but grow out into long chains which often assume spiral arrangements. (Figure 52.)

Spore formation. Spores are formed slowly seldom appearing before 48 hours. They are cylindrical, thicker than the rods from which they spring, terminal or sub-terminal, giving the organisms a clavate or club-shaped appearance. Free spores are 0.75 by 1.5 microns in dimensions.

Motility. Active motility in 24 hour cultures.

Staining properties. Gram-negative.

Agar slant. Thin spreading smooth glistening growth with gradual darkening of the agar.

Agar stab. Faint growth along line of puncture and on the surface at the point of inoculation.

Agar colonies. Colonies grow slowly appearing only after 3 to 4 days. They are round, regular, under low power showing central nuclei with thin spreading peripheries. Deep colonies apt to be irregular under low power, showing clean-cut or entire edges.

Glucose agar. Faint white filmy growth with an alkaline reaction.

Glucose agar colonies. Thin slow-growing spreading surface colonies, under low power showing dense central nuclei and thin margins. Deep colonies punctiform, under low power slightly granular with irregular margins. Reaction alkaline.

Gelatin stab. Growth along line of inoculation and slow cup-shaped liquefaction.

Gelatin colonies. Colonies on the surface show dense central nuclei and concentric spreading peripheral margins. Deep colonies punctiform and tend to show same arrangement. Under low power edges entire.

Broth. Slight turbidity. No scum. No sediment. Fragile scum described by Flügge.

Peptone. Slight turbidity. No scum. No sediment.

Potato. No visible growth in our isolations. Faint moist growth gradually becoming thicker and yellowish, noted by Flügge.

Milk. No change produced by our strains. Slow peptonization described by Flügge.

Blood serum. Thin transparent spreading growth, pale yellow to yellowish-brown. No liquefaction. Slight sinking-in of the growth mentioned by Flügge.

Fermentation tubes. Glucose. Faint turbidity in bowl. No scum. No growth in closed arm. Reaction alkaline.

Saccharose. Appearance the same. Reaction alkaline.

Lactose. Appearance the same. Reaction alkaline.

Thermal death point. Spores survived 10 pounds in autoclave but were killed by 15 pounds pressure.

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ILLUSTRATIONS

The illustrations were drawn by Mrs. W. W. Ford and Mr. W. P. Didusch from preparations illustrating the different phases in the morphology of the various species. The preparations were always stained by gentian violet and drawn from a Zeiss microscope with a 1/12 oil immersion lens and a compensating ocular No. 8, giving a magnification of 1080 diameters. The attempt was made to show the morphology of the vegetative cells which comes out best in certain species at 6 to 7 hours and in others at 22 to 24 hours, the method of spore-formation which varies in the time in which it appears, and the changes which occur in the organism when grown on carbohydrate media.

EXPLANATION OF PLATES

PLATE 1

FIG. 1. *Bacillus coli*.   Plain agar, 24 hours
FIG. 2. *Bacterium anthracis*.   Plain agar, 24 hours

PLATE 2

FIG. 3. *Bacterium anthracis*.   Plain agar, 4 days
FIG. 4. *Bacillus subtilis* from milk.   Plain agar, 20 hours

PLATE 3

FIG. 5. *Bacillus subtilis* from milk.   Glucose agar, 24 hours
FIG. 6. *Bacillus vulgatus* from milk (*Bacillus mesentericus vulgatus*).   Plain agar, 20 hours

PLATE 4

FIG. 7. *Bacillus vulgatus* from milk (*Bacillus mesentericus vulgatus*).   Glucose agar, 24 hours
FIG. 8. *Bacillus mesentericus* from soil (*Bacillus mesentericus fuscus*).   Plain agar, 72 hours

PLATE 5

FIG. 9. *Bacillus mesentericus* from soil (*Bacillus mesentericus fuscus*).   Glucose agar, 48 hours
FIG. 10. *Bacillus aterrimus* from human intestinal contents (*Bacillus mesentericus niger*).   Plain agar, 20 hours

PLATE 6

FIG. 11. *Bacillus aterrimus* from human intestinal contents (*Bacillus mesentericus niger*).   Glucose agar, 48 hours
FIG. 12. *Bacillus niger* from Kral (*Bacillus lactis niger*).   Plain agar, 48 hours

PLATE 7

FIG. 13. *Bacillus niger* from Kral (*Bacillus lactis niger*).   Glucose agar, 48 hours

FIG. 14. *Bacillus globigii* from Kral (*Bacillus mesentericus ruber*).   Plain agar, 20 hours

PLATE 8

FIG. 15. *Bacillus cohaerens* from milk.   Plain agar, 7 hours
FIG. 16. *Bacillus cohaerens* from soil.   Plain agar, 6 hours

PLATE 9

FIG. 17. *Bacillus cohaerens* from soil.   Plain agar, 24 hours
FIG. 18. *Bacillus simplex* from Kral.   Plain agar, 5 hours

PLATE 10

FIG. 19. *Bacillus simplex* from Kral.   Plain agar, 20 hours
FIG. 20. *Bacillus simplex* from soil.   Plain agar, 24 hours

PLATE 11

FIG. 21. *Bacillus simplex* from soil.   Plain agar, 3 days
FIG. 22. *Bacillus mycoides* from cow dung.   Plain agar, 5 hours

PLATE 12

FIG. 23. *Bacillus mycoides* from cow dung.   Plain agar, 24 hours
FIG. 24. *Bacillus mycoides* from cow dung.   Plain agar, 5 days

PLATE 13

FIG. 25. *Bacillus cereus* from milk.   Plain agar, 7 hours
FIG. 26. *Bacillus cereus* from milk.   Plain agar, 24 hours

PLATE 14

FIG. 27. *Bacillus cereus* from milk.   Glucose agar, 24 hours

FIG. 28. *Bacillus albolactus* from milk (*Bacillus lactis albus*).   Plain agar, 7 hours

PLATE 15

FIG. 29. *Bacillus albolactus* from milk (*Bacillus lactis albus*).   Plain agar plate, 24 hours

FIG. 30. *Bacillus albolactus* from milk (*Bacillus lactis albus*).   Glucose agar, 24 hours

PLATE 16

FIG. 31. *Bacillus megatherium* from American Museum.   Plain agar, 7 hours

FIG. 32. *Bacillus megatherium* from American Museum.   Plain agar (plate), 24 hours

PLATE 17

FIG. 33. *Bacillus megatherium* from American Museum.   Glucose agar, 24 hours

FIG. 34. *Bacillus megatherium* from American Museum.   Glucose agar, 48 hours

PLATE 18

FIG. 35. *Bacillus megatherium* from Kral.   Plain agar, 7 hours
FIG. 36. *Bacillus megatherium* from Kral.   Plain agar, 24 hours

PLATE 19

FIG. 37. *Bacillus megatherium* from Kral.   Glucose agar, 20 hours
FIG. 38. *Bacillus petasites* from milk.   Plain agar, 7 hours

PLATE 20

FIG. 39. *Bacillus petasites* from milk.   Plain agar, 20 hours
FIG. 40. *Bacillus petasites* from milk.   Plain agar, 48 hours

PLATE 21

FIG. 41. *Bacillus petasites* from Kral.   Plain agar, 7 hours
FIG. 42. *Bacillus petasites* from Kral.   Plain agar, 20 hours

PLATE 22

FIG. 43. *Bacillus graveolens* from Kral.   Plain agar, 7 hours
FIG. 44. *Bacillus graveolens* from Kral.   Plain agar, 20 hours

PLATE 23

FIG. 45. *Bacillus tumescens* from Kral.   Plain agar, 7 hours
FIG. 46. *Bacillus tumescens* from Kral.   Plain agar 20 hours

PLATE 24

FIG. 47. *Bacillus tumescens* from Kral.   Glucose agar, 48 hours
FIG. 48. *Bacillus fusiformis* from dust.   Plain agar, 24 hours

PLATE 25

FIG. 49. *Bacillus fusiformis* from dust.   Plain agar, 48 hours, showing long threads

FIG. 50. *Bacillus fusiformis* from contaminated hirudin.   Plain agar, 24 hours

PLATE 26

FIG. 51. *Bacillus fusiformis* from contaminated hirudin.   Plain agar, 48 hours
FIG. 52. *Bacillus terminalis* from milk.   Plain agar, 17 days
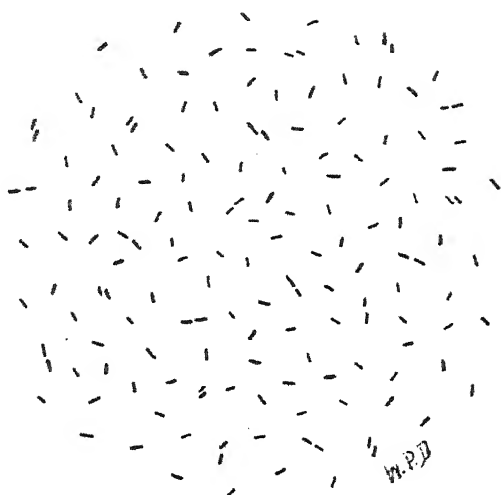
Fig. 1
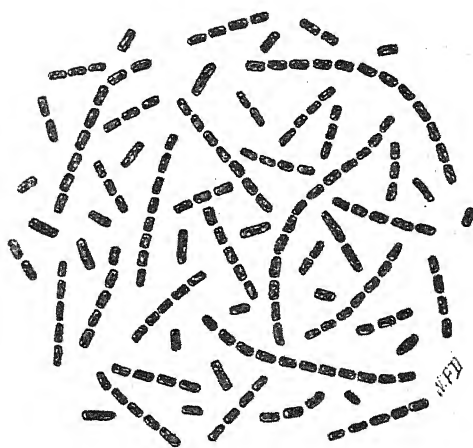
Fig. 2

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
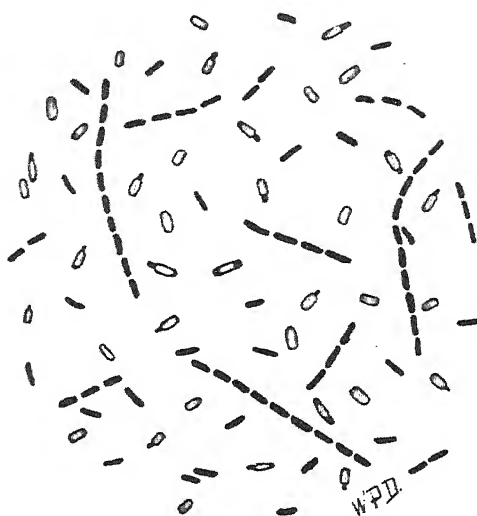
FIG. 3

FIG. 4

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 5

FIG. 6

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
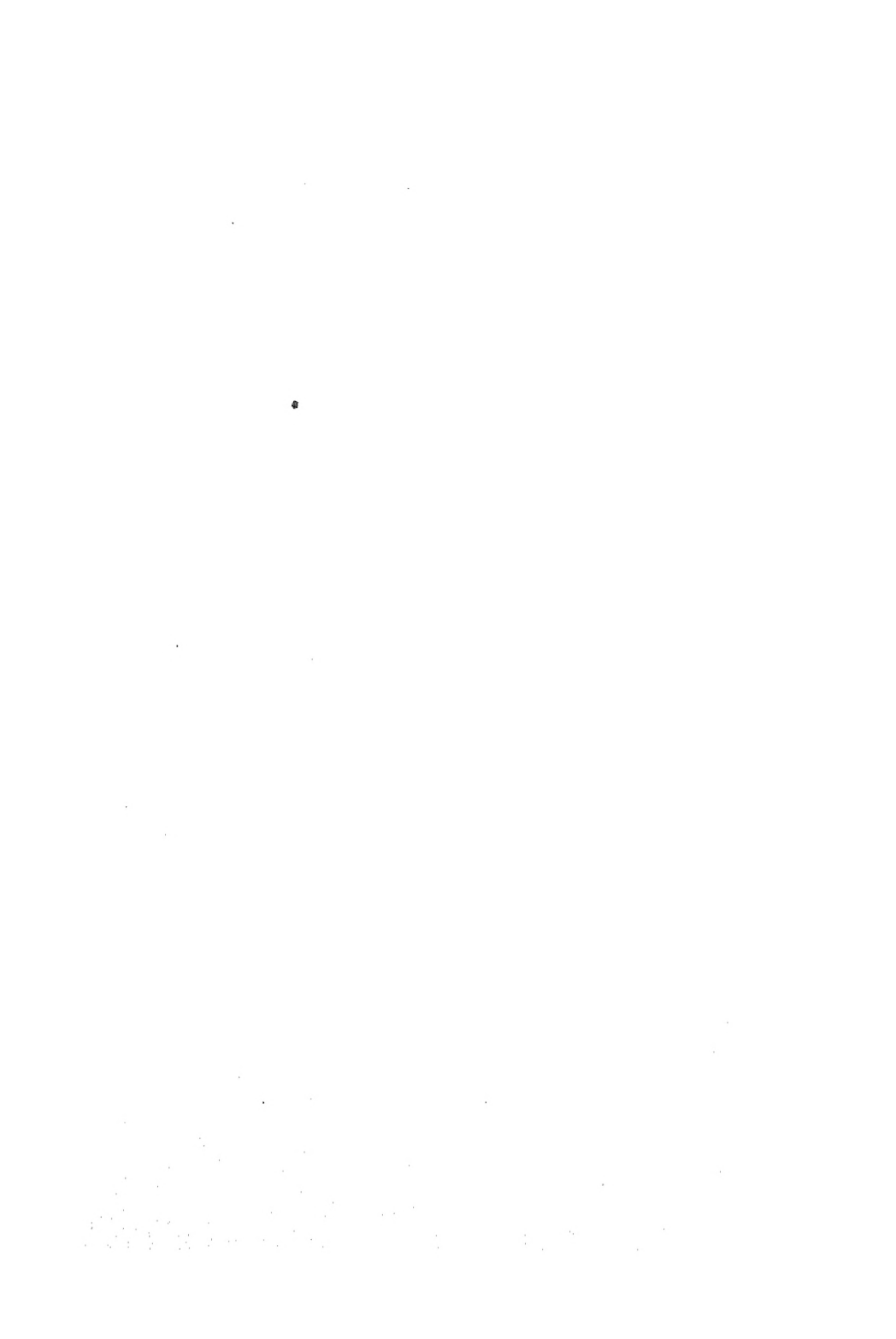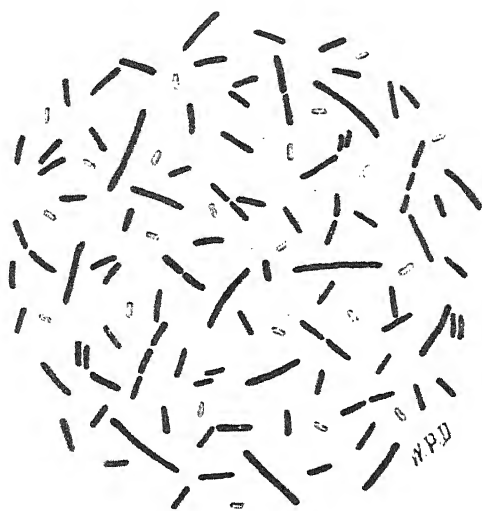
FIG. 7
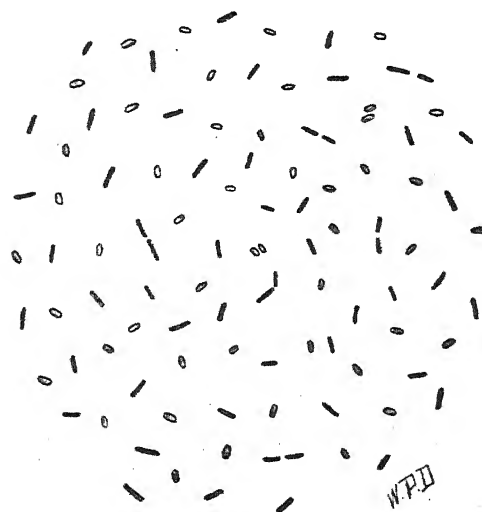
FIG. 8

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
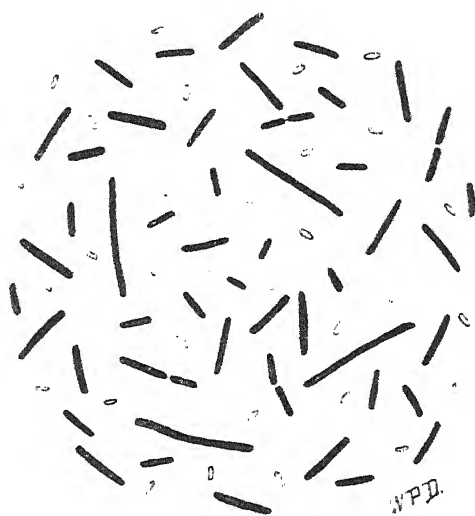
FIG. 9
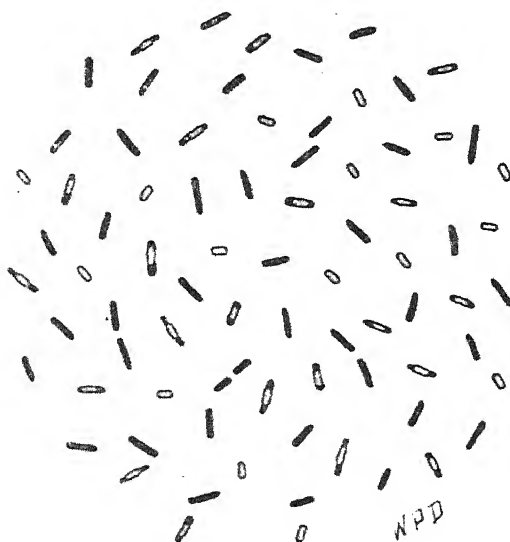
FIG. 10
(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
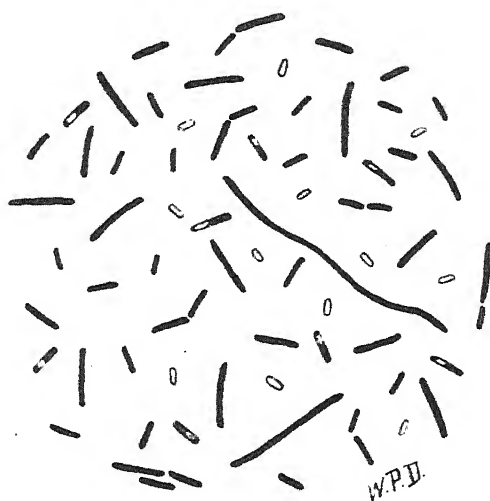
FIG. 11
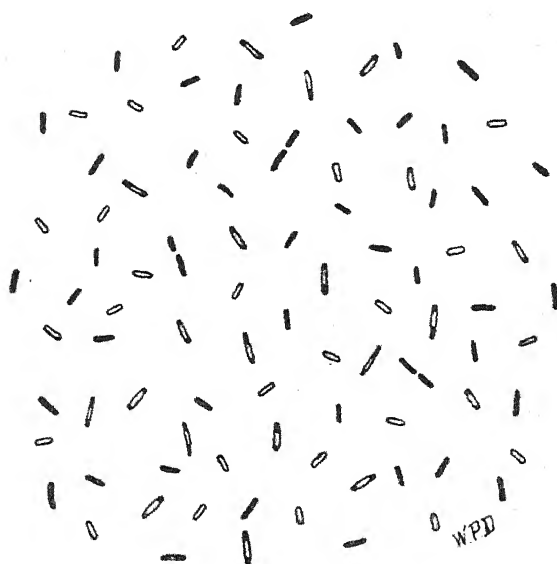
FIG. 12
(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
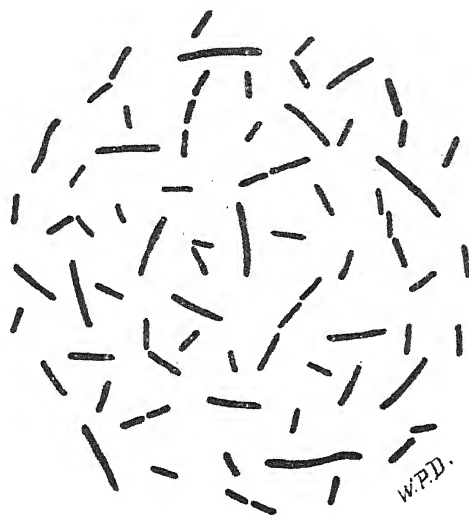
Fig. 13
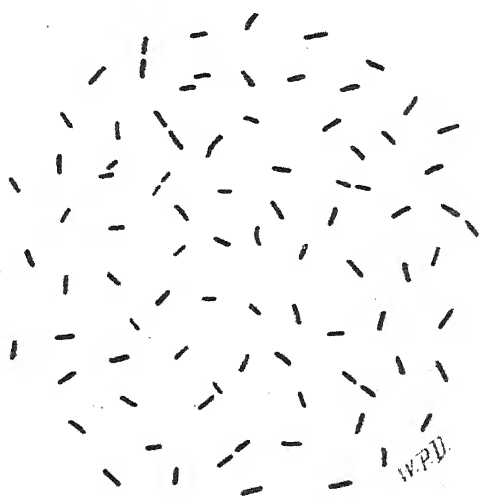
Fig. 14

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
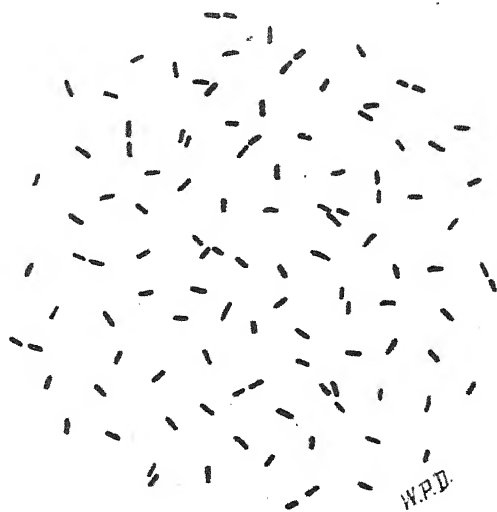
FIG. 15
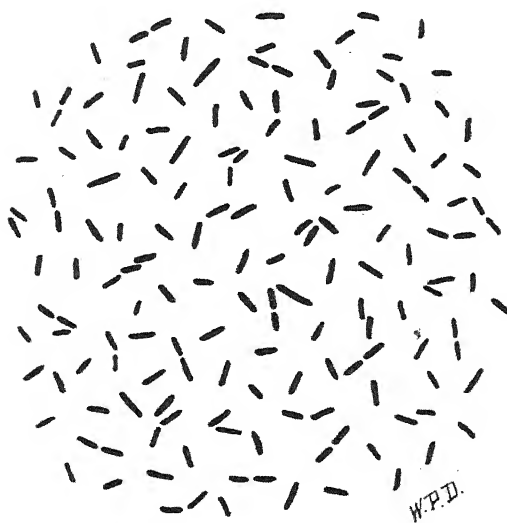
FIG. 16

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
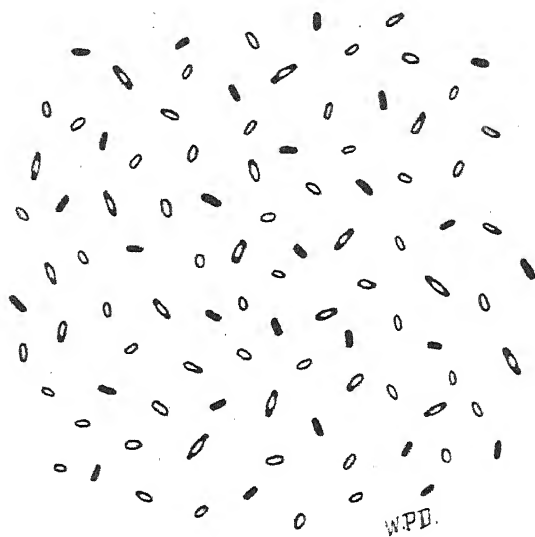
FIG. 17
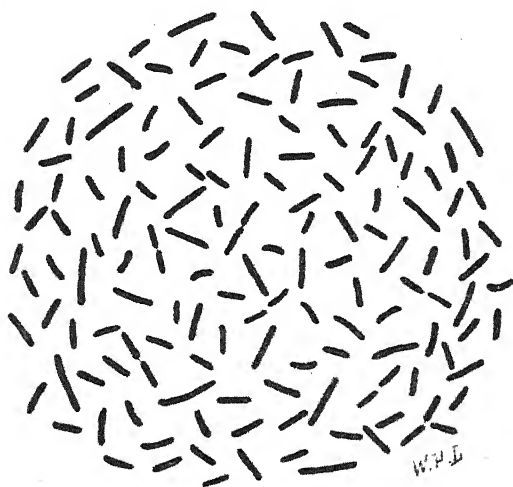
FIG. 18

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 19
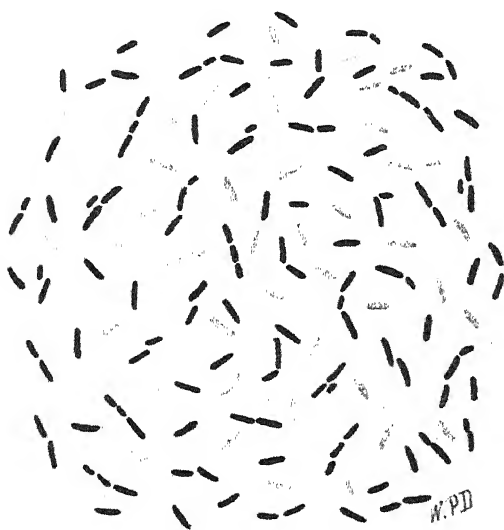
FIG. 20

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
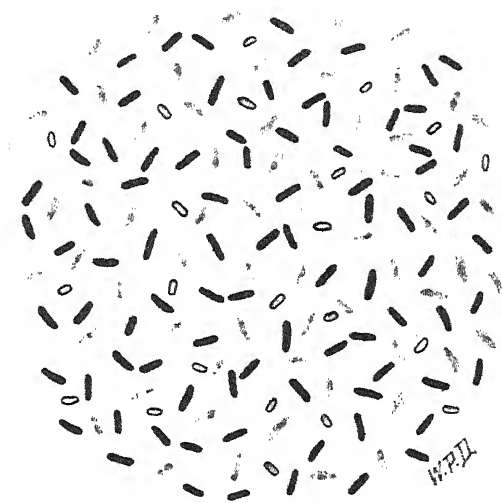
Fig. 21
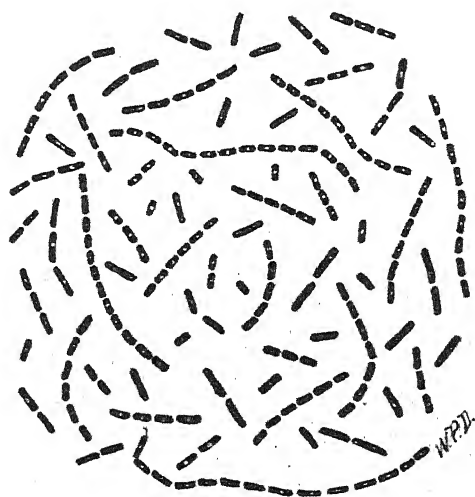
Fig. 22

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 23

FIG. 24

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

PLATE 13
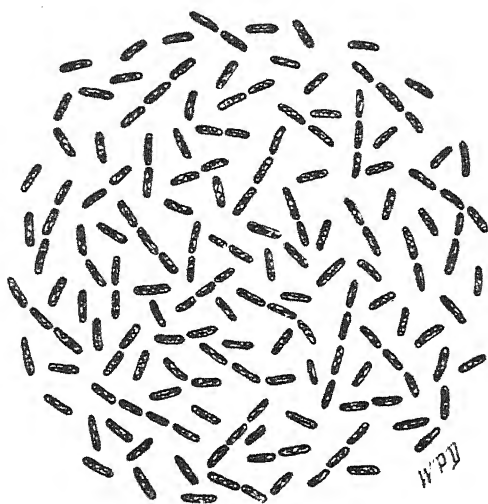
FIG. 25
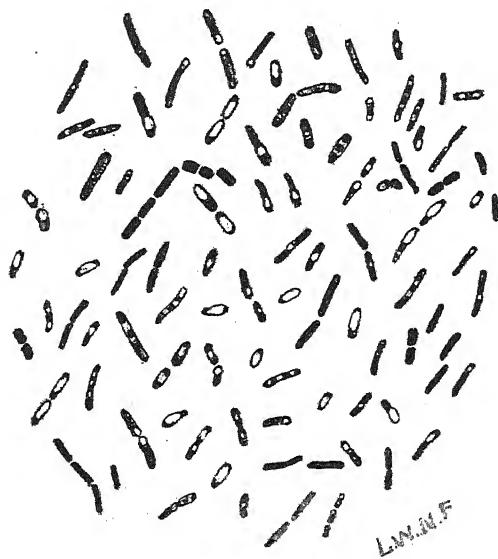
FIG. 26

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

Fig. 27

Fig. 28

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
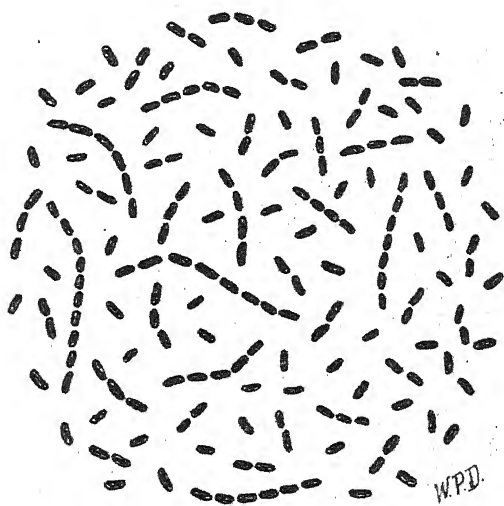
FIG. 29
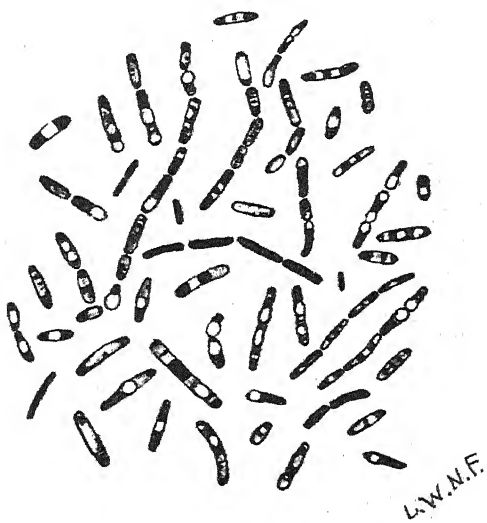
FIG. 30

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
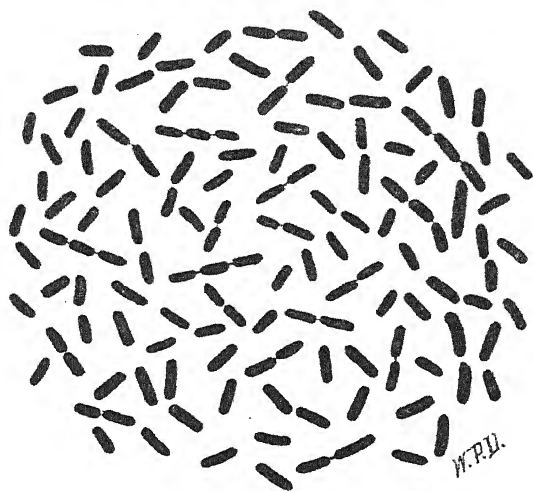
Fig. 31

Fig. 32

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

PLATE 17

FIG. 33

FIG. 34

Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
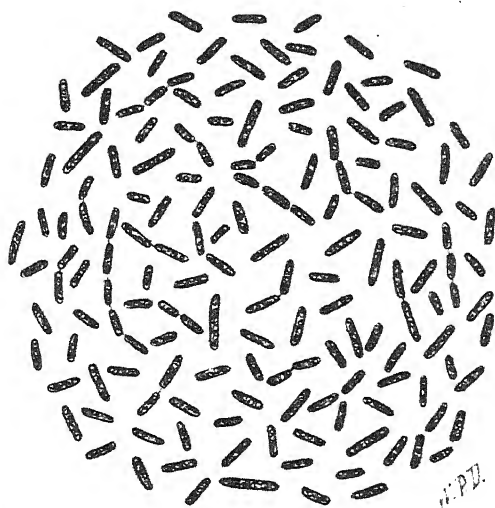
FIG. 35

FIG. 36

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

PLATE 19
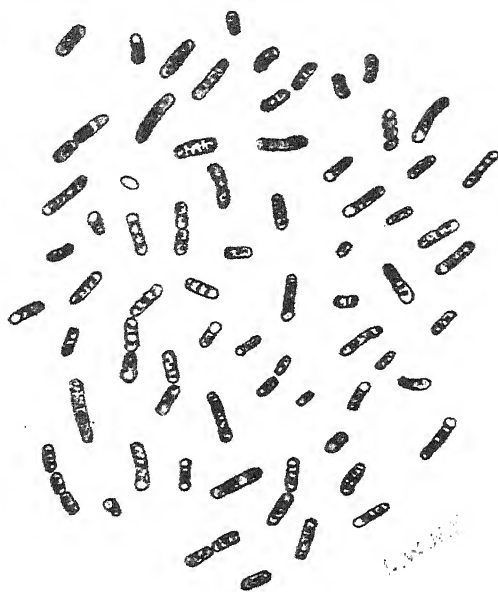
FIG. 37
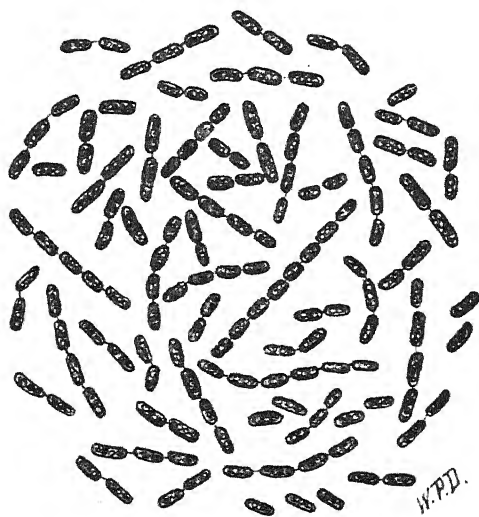
FIG. 38

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
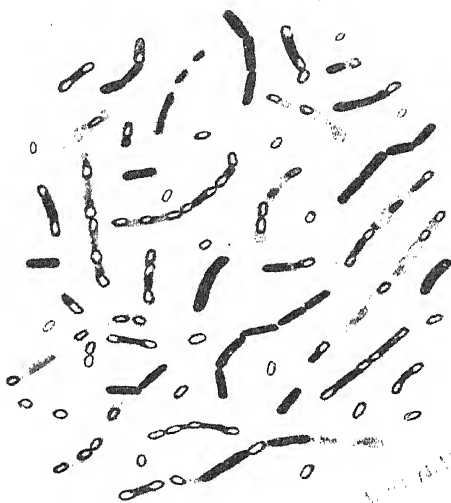
Fig. 39
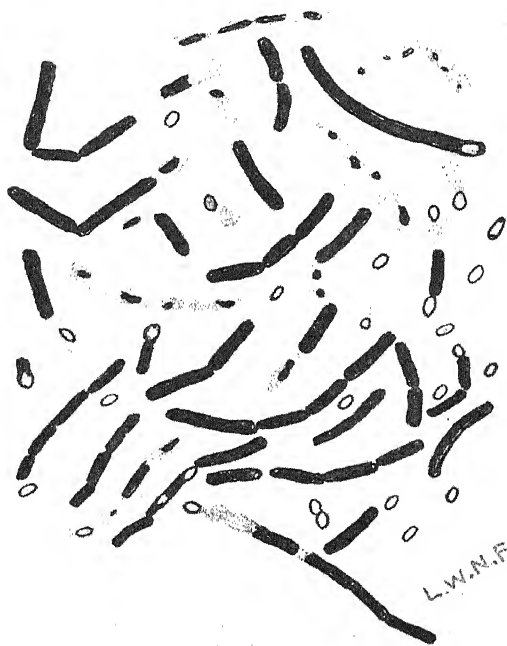
Fig. 40

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
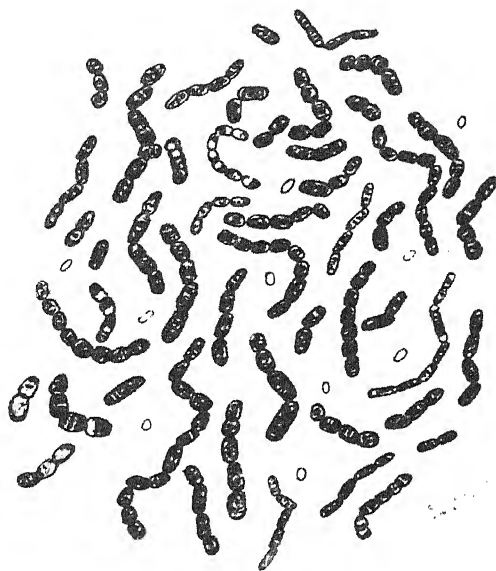
FIG. 41

FIG. 42

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
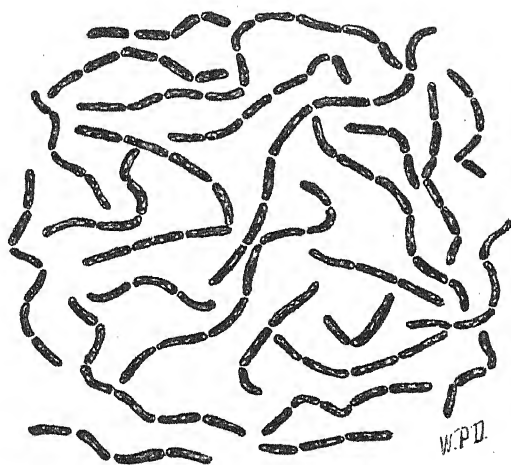
Fig. 43

Fig. 44

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

PLATE 23
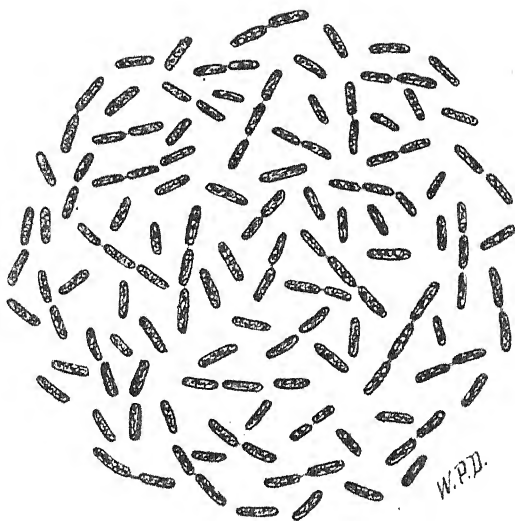
Fig. 45

Fig. 46

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

Fig. 47
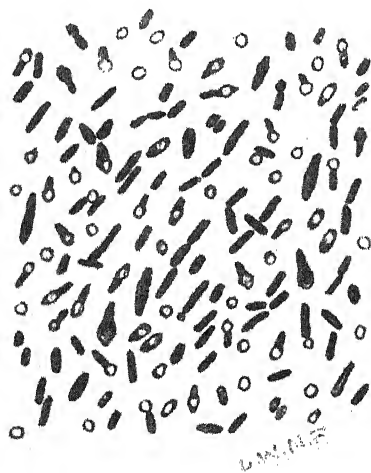
Fig. 48

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

Fig. 49

Fig. 50

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria

PLATE 26
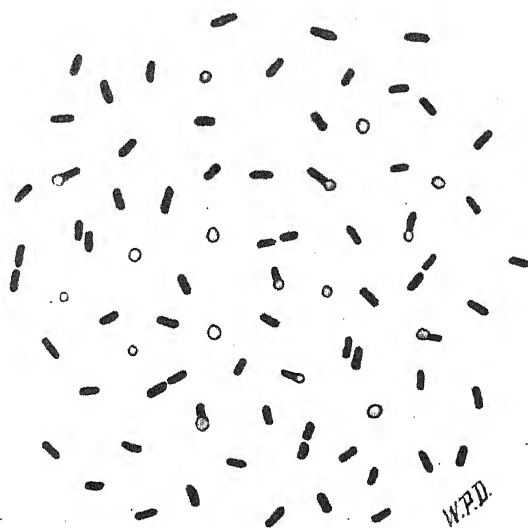
FIG. 51
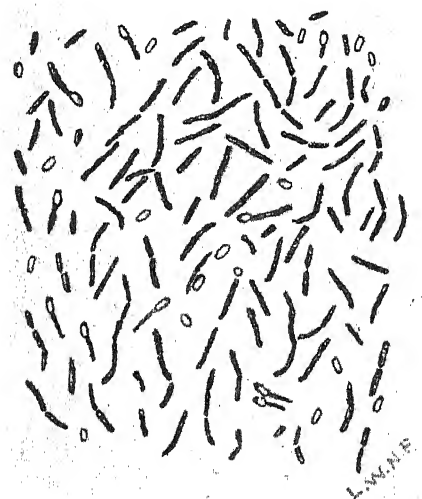
FIG. 52

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

THE NUMBER OF COLONIES ALLOWABLE ON SATISFACTORY AGAR PLATES

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New York Agricultural Experiment Station, Geneva, New York

INTRODUCTION

A point which is of much importance in making bacteriological counts is the limit in the number of colonies that may be allowed to grow on a plate without introducing serious errors. Probably every bacteriological worker has this point in mind in making counts and has his own opinion based on experience; but there are few published data on the subject. The matter has been specially under discussion in connection with the proposed revision of standard methods of milk analysis. This investigation was made in order to increase the amount of information available for the use of the Committees who have undertaken the work of revision.

HISTORICAL

It is interesting to note the published opinions of different workers on this point. In 1895 Neisser (1895) published an article in which he reached the conclusion that plates should be so made that they will have about 10,000 colonies per plate, which numbers should then be estimated by means of the low power lenses of a compound microscope. He undoubtedly believed that each bacterial cell put into an agar plate would produce a colony regardless of overcrowding. Three years later Hesse and Niedner, (1898) realizing, to some extent at

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[1] The senior author of this paper is responsible for the original suggestion of this problem, for direction in carrying it out, and aid in preparing the esults for publication. The junior author has carried out the laboratory work and has helped in preparing the results for publication.

least, the true state of affairs published an article in which they claim that plates having more than 100 colonies should be disregarded and that under these conditions the microscope should not be used for counting. In 1897, Hill (Hill and Ellms, 1897) contended that overcrowded plates would not give reliable results in water analysis. In 1899, Jordan and Irons (1899) independently urged the same thing. Again Hill (1908) called attention to the point in a paper read before the laboratory section of the American Public Health Association in 1907, in which he pointed out that wide discrepancies in counts might be caused by different methods of computation and concluded that only those plates having numbers of colonies falling between 40 and 200 per plate should be considered in reporting results. These figures were adopted in the report presented by the Committee on Standard Methods for the Bacterial Examination of Milk at the Richmond meeting of the American Public Health Association (1910). In the Report presented at the Rochester meeting in September 1915 (Comm. Stand. Meth. 1915) the lower limit in the number of colonies allowable on agar plates was changed from 40 to 30, and the limits of 30 and 200 were also accepted by the Committee on Standard Methods of Bacterial Water Analysis in their Report presented at the same meeting.

STATEMENT OF PROBLEM

It is generally recognized that the kind of bacteria present in the material under examination will have an influence on the size of the colonies, and, consequently, on the number that can develop on a plate. Some of the commonest and most important bacteria in milk do not produce colonies larger than pin points on ordinary agars even when only a few are present. Other colonies grow large and in the case of spreaders may cover the entire plate.

Just what prevents the development of all the bacteria into colonies on crowded plates is not thoroughly understood. In some cases it may be because the food material is all used up;

in others it is clearly due to the fact that by-products of bacterial growth inhibit the growth of other colonies; and occasionally colonies fuse or overgrow each other and so reduce the count. On the other hand colonies growing side by side sometimes stimulate each other, a phenomenon which has been noted in this work on plates containing large numbers of *B. bulgaricus* with an occasional mold or bacterial colony of a different type. The molds and many bacteria so stimulate the *B. bulgaricus* that these organisms form visible colonies in the region of the larger colonies, failing to develop in all other parts of the plate. The same condition has been noted in plating material containing large numbers of long chained streptococci. This phenomenon naturally produces marked irregularities in count when it occurs.

Because of these and other difficulties certain plates in any series made from a given sample are more satisfactory for use in computing a total count than are others. The matter of selecting plates to be used in computing a count becomes therefore a matter requiring considerable judgment.

EXPERIMENTAL DATA

a. Analyses made in the Station Laboratory

The object of this study has been to determine the limits in the number of colonies on plates which are satisfactory for making bacterial counts. The data used have been obtained by plating market milk samples on standard agar in triplicate and in three different dilutions, incubating for five days at 21°C., following with an incubation for two days at 37°C. The plates were counted at the end of five days and again after the two days incubation at 37°C. The five day and seven day counts are tabulated separately and show the conditions for each period of incubation.

In deciding which plate counts to select as probably nearest correct it became necessary to discard all of the counts on a few samples where no satisfactory average could be made because of spreaders or because the milk contained more bacteria

than was anticipated and the dilutions were not carried far enough to give assurance that the count was not affected by overcrowding. In selecting individual plate counts which were to be tabulated as satisfactory, those counts were chosen which could be used in making an average without any individual figure varying more than 20 per cent from the average. All others were listed as discrepancies. For example, one sample gave the following counts per plate, 1: 100 dilution 1944, 1472 and 1928 colonies; 1: 1000 dilution 484, 515 and 610 colonies; 1: 10000 dilution 43, 45, and 46 colonies. The counts of 484 and 515 from the 1: 1000 dilution were averaged with the 1: 10000 counts of 43, 45 and 46; and this average was taken as the final count on the sample. The counts made on the 1: 100 plates were all listed as discrepancies because they are more than 20 per cent lower than the average, and the count of 610 from one of the 1: 1000 plates was also listed as a discrepancy because it was more than 20 per cent higher than the average. Occasionally all of the nine plates made from a sample could be included in the final average.

Table I gives the number of plate counts made after five days of incubation at 21°C., arranged in groups according to the number of colonies which appeared on the plates. Four hundred and thirty-nine of the 1435 plates had less than 10 colonies per plate. Only 22.3 per cent of these checked within the 20 per cent limit. One hundred and eighty plates fell in the group having more than 10 and less than 20 colonies per plate. Of these 53.9 per cent checked within the 20 per cent limit. Percentages calculated for the groups of plates having 20 to 30, 30 to 50, 50 to 100, 100 to 200 and 200 to 400 colonies per plate were more or less variable, showing that from 66.3 per cent to 93.2 per cent of the total number of plates agreed within the 20 per cent limit. The best percentage of agreement is shown by the group having more than 100 and less than 200 colonies per plate, and the next highest by the group having between 50 and 100 colonies per plate. There were decidedly fewer plates giving satisfactory results among those which had more than 400 colonies per plate, the percentage of plates which checked within 20 per cent being 44.4.

The results given in the lower part of table 1 were calculated from the same counts, the groups of plates having been arranged differently. From this part of the table it will be seen that the percentage of discrepant plates is practically the same for the groups of plates having 20 to 400, 30 to 400, 20 to 200, 30 to 200, or 40 to 200 colonies per plate, the best showing being made by the group of plates having more than 40 and less than

TABLE 1

Plate counts after incubation at 21° C. arranged to show the number and percentage of counts in groups according to the number of colonies per plate

GROUP	CHECKED WITHIN 20 PER CENT OF AVERAGE		DISCREPANT PLATES, DID NOT CHECK WITHIN 20 PER CENT OF AVERAGE				TOTAL NUMBER OF PLATES IN GROUP
	Number	Per cent	Too low	Too high	Total number	Per cent	
0 to 10	98	22.3	172	169	341	77.7	439
10 to 20	97	53.9	29	54	83	46.1	180
20 to 30	54	72.9	6	14	20	27.1	74
30 to 50	67	66.3	11	23	34	33.7	101
50 to 100	162	84.8	17	12	29	15.2	191
100 to 200	179	93.2	8	5	13	6.8	192
200 to 400	105	78.9	25	3	28	21.1	133
Over 400	100	44.4	114	11	125	55.6	225
0 to 30	249	35.9	207	237	444	64.1	693
20 to 400	567	82.0	67	57	124	18.0	691
30 to 400	513	83.1	61	43	104	16.9	617
20 to 200	470	82.9	43	54	97	17.1	567
30 to 200	416	84.3	37	40	77	15.7	493
40 to 200	376	86.0	23	28	61	14.0	437
Over 400	100	44.4	114	11	125	55.6	225

Total number of counts summarized in this table 1435.

200 colonies per plate. Plates having less than 30 colonies or more than 400 colonies show very large percentages of discrepancies.

Table 2 gives the results obtained by counting 1056 of the same plates as those whose counts are summarized in table 1 after two days of additional incubation at 37°C. In general the results obtained from these counts are similar to those given in table 1. However the best showings are made in this case by groups of plates having more than 200 and less than 400 colo-

nies per plate (87 per cent of satisfactory plates), the group of plates having 100 to 200 colonies (82.4 per cent) and the group having 30 to 400 colonies per plate (81.4 per cent). As in table 1 there is a marked increase in the number of discrepant counts from plates having less than 30 or more than 400 colonies per plate While the results in table 1 favor the 40 to 200 group rather than the 30 to 400 group by 2.9 per cent., the same

TABLE 2

Plate counts after two additional days of incubation at 37° C. arranged to show the number and percentage of counts in groups according to the number of colonies per plate

GROUP	CHECKED WITHIN 20 PER CENT OF AVERAGE		DISCREPANT PLATES, DID NOT CHECK WITHIN 20 PER CENT OF AVERAGE				TOTAL NUMBER OF PLATES IN GROUP
	Number	Per cent	Too low	Too high	Total number	Per cent	
0 to 10	60	28.4	60	91	151	71.6	211
10 to 20	76	60.0	23	28	51	40.0	127
20 to 30	46	63.0	8	19	27	37.0	73
30 to 50	55	72.3	5	16	21	27.7	76
50 to 100	117	81.0	14	12	26	19.0	143
100 to 200	127	82.4	16	11	27	17.6	154
200 to 400	101	87	14	1	15	13	116
Over 400	78	50	74	4	78	50	156
0 to 30	182	44.2	91	138	229	55.8	411
20 to 400	445	79.2	57	61	117	20.8	562
30 to 400	399	81.4	49	42	91	18.6	490
20 to 200	353	77	45	60	105	23	458
30 to 200	307	79.7	37	41	78	20.3	385
40 to 200	277	79.8	36	34	70	20.2	347
Over 400	78	50	74	4	78	50	156

Total number of counts summarized in this table 1056.

comparison in table 2 shows an advantage of 1.6 per cent for the 30 to 400 group   This indicates that there is little advantage in selecting one group of plates in preference to the other.

In the fourth and fifth columns of these two tables, the number of cases is shown in which the discrepancy was caused by having too few or too many colonies on the plate Arranging the plates in the groups 0 to 10, 10 to 20, 20 to 30, 30 to 50, 50 to 100, 100 to 200, 200 to 400 and more than 400 colonies per

plate, it is seen that there is a tendency for discrepancies caused by having too many colonies on a plate to occur in all groups having less than 50 colonies per plate (one exception to this statement is seen in the group 0 to 10 in table 1). In all cases where more than 50 colonies occurred on the plates, the greater number of discrepancies was caused by having too few colonies on the plates. The tendency toward discrepancies caused by having too few colonies on the plates becomes very marked as soon as the limit of 200 colonies per plate is passed.

These findings indicate that while the greater proportion of the discrepancies on plates having less than 50 colonies per plate are caused by the operations of the laws of choice and chance, yet there is some factor present which tends to cause more colonies to develop than should do so. In all probability this factor is chance contamination from the air which occurs during planting. As is well known, it is common for supposedly sterile check plates to develop one, two or more colonies on prolonged incubation. The presence of these colonies on inoculated plates having fewer than 50 colonies per plate causes a relatively large error in the counts which in some cases would cause the individual plate count to exceed the 20 per cent limit specified here as necessary before the plates were classed as satisfactory.

The tendency for irregularities, due to having too few colonies on plates, to occur in counts having 50 or more colonies per plate is too well known to all bacteriologists to require extended discussion. These are undoubtedly caused by the effect of overcrowding. The fact that not all of the discrepancies on plates having more than 400 colonies per plate were of this sort is more significant, for it shows that not all of the discrepancies on plates having numerous colonies are due to overcrowding. Irregularities in the number of bacteria used in inoculating or chance contaminations are two things which might produce plates having too many colonies even on crowded plates.

When all of these things are taken into consideration, it becomes a difficult matter to decide upon the limits in number of colonies which should be allowed on plates. It is at once

clear that plates having less than 20 and more than 400 colonies are so apt to be widely discrepant that counts from plates of this sort should be disregarded. There are likewise clear indications that plates having between 40 and 200 colonies per plate are as satisfactory as any that can be selected. However the results secured in this investigation do not indicate that serious errors would be introduced in routine work by extending these limits to 30 and 400, or even to 20 and 400, thereby lessening the amount of work necessary to secure acceptable counts.

b. New York City analyses

Another set of data which is more satisfactory in one way because of the fact that a very large number of plates were made from a single sample of milk but which is also less satisfactory in another way because of the fact that it is more limited in its application, has been secured from a set of analyses made on November 19, 1915, by five New York State laboratories,[2] under the supervision of Prof. H. W. Conn. In this series 20 samples of the same milk were sent to each laboratory for analysis. Four laboratories made plate counts, one making them in duplicate, so that five sets of plate counts are available. These were made from two dilutions of 1: 100 and 1: 1000 each. Two plates were made for each dilution. Three laboratories made microscopic counts, one making them in duplicate so that four sets of these counts are available.

The average of the accepted plate counts was 4250. The average of the microscopic counts of clumps, or sources, was 5590. The close correspondence in results obtained by these two very different methods of counting makes it very probable that the total number of groups of bacteria in this milk was close to 5000 per cubic centimeter. The 1: 100 dilution plates gave counts in which the average number of colonies on the two plates varied between 24 and 125. The 1: 1000 plates gave counts in which the average number of colonies from the two plates varied be-

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[2] Lederle Laboratories, North's Sanitary Laboratories, N. Y. City Board of Health Laboratory, Borden's Laboratory, N. Y. Agric. Exp. Sta. Laboratory.

tween 0.5 and 16.5 with a single case where the average of the two plates was 44.

If we arbitrarily assume that plates giving a count more than 2500 above or below the average fail to check with the accepted count, we find that the averages of all but three of the 100 pairs of 1: 100 plates check with the accepted count while there are 27 cases out of the 100 where the count from the 1: 1000 dilution fails to check within these limits. It is important to note also that 23 of these 27 cases are instances where the discrepancy was such as to give a higher count that the accepted count, indicating that chance contaminations were probably the chief cause of trouble.

SUMMARY

1. The work here reported includes a study of the counts made from 1435 agar plates inoculated from samples of market milk and incubated five days at 21°C.; and also a study of the counts made from 1056 of the same plates after two days additional incubation at 37°C. The results obtained indicate that, for milk analyses, the counts made from plates having more than 30 and less than 400 colonies on the plates are very nearly as satisfactory as those obtained from plates having more than 40 and less than 200 colonies, the latter being the limits in numbers originally recommended by the Committee on Standard Methods for the Bacterial Examination of Milk.

2. Plates having less than 20 or more than 400 colonies on them are shown to be so frequently discrepant that counts obtained from them should never be trusted unless checked by comparison with plates from different dilutions having more than 30 or less than 400 colonies. The acceptance of counts from plates having 20 to 30 colonies per plate would not greatly increase the percentage of discrepancies.

3. All groups of plates, regardless of the number of colonies showed a certain percentage of plates which gave counts which varied more than 20 per cent from the accepted count. The percentage of discrepant counts of this sort varied between 37

and 7 for all groups of plates having more than 20 and less than 400 colonies per plate, the worst showing being made by the plates having 20 to 30 colonies per plate and the best by the plates having 100 to 200 colonies per plate.

4. The discrepancies which occurred in counts made from plates having less than 50 colonies per plate were more frequently caused by too many colonies on the plates than by too few colonies. This excess is undoubtedly due to the influence of chance air contaminations which took place during the plating. Where the plates have a small number of colonies on them a few extra colonies of this sort produce relatively wide discrepancies.

5. The discrepancies in counts made from plates having more than 50 colonies per plate were more frequently caused by having too few rather than too many colonies on the plates. The frequency of this type of discrepancy became very marked where the number of colonies exceeded 200 per plate. The probable explanation of the excess of this type of irregularity is that of overcrowding. Since however there was always a certain percentage of discrepancies caused by having too many colonies on the plate even where there were more than 400 colonies per plate, it is evident that not all of the irregularities are caused in this way.

6. Counts made from 20 duplicate samples of the same milk in five series of analyses showed 27 out of a possible 100 wide discrepancies in the counts obtained from an average of two plates made from a 1: 1000 dilution. The number of colonies of these plates averaged more than 0.5 and less than 16.5 for the two plates, with one exception where the average was 44. Counts made from the 100 pairs of 1: 100 plates which had more than 24 and less than 125 colonies as the average of the two plates, showed only 3 out of a possible 100 wide discrepancies.

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A MODIFICATION OF THE HYGIENIC LABORATORY METHOD FOR THE PRODUCTION OF TETANUS TOXIN

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In the November, 1915, issue of the Journal of Medical Research,[1] Anderson and Leake briefly describe the method used at the Hygienic Laboratory, Washington, D. C., for the production of a uniformly potent toxin. The method in use at the Research Laboratory is essentially that given by Anderson and Leake with a few slight variations. As there have been many inquiries as to how we obtain our potent toxin it was thought that the details of the exact procedure might be of sufficient value to publish.

STOCK CULTURES

The stock cultures are grown on a semi-solid medium made in the following way.

Veal broth	1000 cc.
Agar	5 grams
Witte's Peptone	10 grams
NaCl	5 grams
Reaction	Neutral to phenolphthalein

About 8 cc. to 10 cc. of this medium are put into tubes which are autoclaved at 15 pounds pressure for one-half hour, the tubes being then ready for use.

To transfer cultures, one of the semi-solid agar cultures is melted and 1 cc. is added to a freshly melted semi-solid agar tube, at least ten sub-cultures being thus made from one stock culture. After inoculation, the tubes are cooled, the plugs immersed in

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[1] Anderson and Leake, 1915. A Method of producing Tetanus Toxin, Journal of Medical Research, **33**, 239.

paraffin and the tubes incubated at 37°C. After one week's incubation the cultures are stored in the ice chest, where they may be kept for six months without affecting their ability to produce toxin.

THE BROTH FOR TOXIN AND PRELIMINARY CULTURES

The following broth is used both for the toxin and for the preliminary cultures for inoculating the toxin broth:

To every pound of market veal, add 1000 cc. of water and place in ice chest over night. The next morning the infusion is placed over a free flame and raised to 45°C. and held at that temperature for one hour; it is then boiled briskly for one-half hour and the broth strained through cheesecloth. The amount of filtrate is measured and the following added:

Witte's Peptone .... 1.0 per cent
Glucose (anhydrous) .... 1.0 per cent
NaCl .... 0.5 per cent

When the above ingredients are added, the broth is boiled until the peptone, glucose, etc., are melted, and is then titrated with $\frac{N}{10}$ NaOH. After the reaction has been corrected to +1 to phenolphthalein, the flame is turned out. The broth is then filtered through two layers of absorbent cotton directly into 2 litre Erlenmeyer flasks, leaving only sufficient space in the flasks for the expansion of the broth during the sterilization in the Arnold. The flasks are sterilized for $1\frac{1}{2}$ hours on the first day and one hour on the second day.

PRELIMINARY CULTIVATION

Fill potato tubes with about 40 cc. of the glucose broth and sterilize for one and one-half hours on the first day and one hour on the second day. These tubes may be kept for two weeks when they will still give satisfactory growth. To make the first transfer for the preliminary cultivation, add 8 cc. of the melted semi-solid agar stock culture of the *B tetani* to two tubes of glucose broth from which the air has been previously

expelled by heating in the Arnold for fifteen to twenty minutes, and which have been cooled down to about 50°C. These tubes are incubated for 24 hours and the next day, two freshly heated tubes of glucose broth are inoculated with 5 cc. of the glucose broth cultures planted the previous day. On the third day, determine the number of flasks that are to be inoculated and inoculate as many freshly heated glucose broth tubes from the second glucose broth generation as there are flasks. Anderson calls for at least six or seven generations in the glucose broth before the inoculation of the toxin broth, but at the Research Laboratory it has been found that three generations or even two if need be, are sufficient for obtaining a toxicity of 1–25,000.

INOCULATION OF TOXIN BROTH

After the second sterilization in the Arnold, the flasks are ready for inoculation. The broth may be cooled down to 55°–60°C. by allowing the flasks to stand at room temperature, or in a more rapid way by placing the hot flasks in a large sink, to which cool, and then cold, water is added until the lower portions of the flasks are covered. When the bottoms of the flasks are cool to the hand, the portions above the water being still very hot, the inoculation may be made as follows:

The plugs are carefully removed, the necks flamed and the plugs replaced. In a similar way, the mouths of the culture tubes are sterilized and then, partly removing the plug of a flask, the contents of a potato tube is poured rapidly into a flask. If one prefers, the broth culture may be transferred by using a pipette, but the former method has been used here without subsequent contamination and found very satisfactory. After inoculation, the flasks are incubated for fifteen days at 36°–37°C., care being taken to exclude all light from them.

The flasks at the end of 24–48 hours show a diffuse cloudiness with the formation of gas bubbles on the surface of the broth. Toward the end of two weeks, the gas bubbles usually disappear, while the cloudiness persists and a light precipitate forms at the bottoms of the flasks. If it is not convenient

to filter on the fifteenth day, the cultures may be kept in the incubator until the twentieth day without a loss of toxicity but from the twentieth day to the twenty-fifth day, the toxin loses about 20 per cent in potency.

FILTRATION

All glassware, filters, etc., should be neutral to phenolphthalein and the greatest care should be taken to exclude light, either direct or indirect, by darkening the room and by covering the filtering apparatus with dark cloths, ordinary black cambric being used at the Research Laboratory.

The broth cultures are first passed through Buchner filters about 8 inches in diameter, which have been packed with a layer of finely shredded paper pulp 0.25 inch in thickness. It is of importance that the pulp should be so well packed that the filtrate is absolutely clear, otherwise it will clog the Berkefeld filter. The first filtrate, about 200 cc., which passes through the Buchner is discarded, as it contains a considerable amount of water from the pulp, and then the filtering of the toxin may proceed. If, after passing 8 to 14 litres through the pulp, the filtrate begins to appear cloudy, the pulp must be discarded and the Buchner repacked. The clear filtrate is then passed through a sterile Berkefeld filter, and 10 per cent of a 5 per cent solution of carbolic acid solution is added to the toxin which is now placed in the ice-chest, ready for testing its potency.

POTENCY TEST

Two 350 gram guinea pigs are inoculated subcutaneously over the abdomen with 1 cc. of a dilution of $\frac{1}{15,000}$ and $\frac{1}{25,000}$ of the toxin respectively. If the toxin has a potency of $\frac{1}{25,000}$ the pig receiving the $\frac{1}{15,000}$ dilution will die on the second to third day and the pig receiving the $\frac{1}{25,000}$ dilution should die on the fourth day. If both pigs die with symptoms of tetanus before the fourth day, the toxin is stronger than $\frac{1}{25,000}$ and a higher dilution should be tested.

Though no comparative tests have been made with toxin produced under the usual anaerobic conditions, the toxin produced by the Hygienic Laboratory method showed a somewhat unexpected stability. For example:

	Filtered	Tested	Toxicity
Lot 25	7–24–14	7–24–14	1–25,000
		8–31–14	1–25,000
		9–30–14	1–20,000
		10– 4–14	1–20,000

The test was made on a small amount of toxin, about 30 cc., kept in an ordinary test tube in the ice-box and protected from the air only by a shallow covering of albolene.

From the above, it will be seen that our method for growing the tetanus bacillus for toxin varies very slightly from that of the Hygienic Laboratory. Instead of beef, we use market veal which has given almost invariably a toxin of $\frac{1}{25,000}$, even going as high as $\frac{1}{40,000}$ occasionally. It is interesting to note that with the bob veal, that is, veal under the legal age limit for selling, which has always given a more highly potent diphtheria toxin than the older veal, we have rarely obtained a tetanus toxin above $\frac{1}{15,000}$ whereas the market veal used as control has produced a toxin of $\frac{1}{25,000}$ strength.[2]

Anderson recommends that the preliminary cultures should be carried on from one to three weeks, by daily transfers, but with only three generations in glucose broth before inoculating the toxin flasks, we have obtained a toxicity of 1–40,000 showing that fewer generations may be sufficient.

Here it might be well to state that we obtained different results from those recorded by other writers in regard to the growth in the preliminary cultures. Anderson and Leake found that a good growth is obtained in the first generation in 48 hours, in 24 hours in the second and third transplants, and in 16 hours

[2] Furthermore, by inoculating the glucose broth directly with a melted semisolid agar culture of *B. tetani*, we have secured a toxin of over $\dfrac{1}{25,000}$ in strength, the control test which had been inoculated from preliminary broth cultures giving the same degree of toxicity. Confirmatory tests are being made along this line.

in the fourth. At the Research Laboratory, we have found an abundant growth in 18 to 24 hours in the first generation in glucose broth from the semi-solid; this profuse growth continues in the subsequent cultures up to the third or fourth generations, when there is a diminution, until in the sixth or seventh, as frequently happened, no growth or very slight growth appears after 48 hours or more. That is, out of thirteen cultures transferred in the sixth generations, there may be only six or seven tubes which show signs of growth, even after several days incubation.

At the Hygienic Laboratory, glucose stab cultures are used for growing the stock strains, but it is not stated whether these cultures are grown anaerobically or aerobically. The semi-solid agar that we are using for the stock cultures is especially satisfactory as no anaerobic conditions except such as the medium provides are necessary to produce a heavy growth of *B. tetani* with spore formation after a few days incubation.

It is in the hope that other laboratory workers may find the above technique of practical aid in producing an uniformly potent toxin that these minute details have been given.

A METHOD OF ANAEROBIC PLATING PERMITTING OBSERVATION OF GROWTH[1]

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Because of the already numerous descriptions of methods for growing cultures anaerobically, one hesitates to add another method to the list without an apology. The method here described will however be found of advantage in the isolation of anaerobes from cheese, milk, soil or other material where it is desired not only to secure growth of the anaerobes, but also to obtain them in pure culture directly from the material under examination. The method is chiefly to be recommended because of the relatively small amount of inert gases necessary to replace the air in contact with the media—an advantage that will be appreciated in laboratories where generous supplies of these gases are not available and where the method of anaerobic plating is frequently employed on a small scale. Furthermore. the rate and character of growth of the colonies are easily observed from the beginning, so that it is not necessary to open the anaerobic chamber at any time until sub-cultures are to be made.

The apparatus consists of one half of a Petri dish sealed with paraffine, of relatively low melting point, on a square stone or metal base provided with an inlet for the inert gas and an outlet for the displaced air. The base is conveniently made as follows: Slabs of stone (such as Alberine stone, or soapstone) or of cast iron, of about 2 cm. thickness, are cut in squares about 1 cm. larger than the diameter of the Petri dish to be used. Cut in one face of these slabs, with a lathe, an annular groove 3 mm. deep and 4 mm. wide, and of such a diameter that the

edges of the Petri dish will fit loosely into this groove. Within
the circle described by this groove, about 2 cm. from an edge
of the slab; i.e., about 5 mm. from the inner edge of the groove,
drill a hole about 4 mm. in diameter and about half the thick-
ness of the slab in depth. Drill a similar hole for the opposite
side of the annular groove. Now drill horizontal holes from the
corresponding surface edges of the slab to meet the vertical
holes, and of such a diameter that small rubber stoppers may be
used to stopper these holes securely. With this slab as a base
for the Petri dish, the method of manipulation, provided it is
desired to grow all of the colonies on the surface of the media,
is somewhat as follows: Pour the agar or gelatin into the Petri
dish as usual and allow to solidify. Then flow suitable water
or broth dilutions of the material to be examined on the surface

Cross Section view of stone base
with petri dish in position.
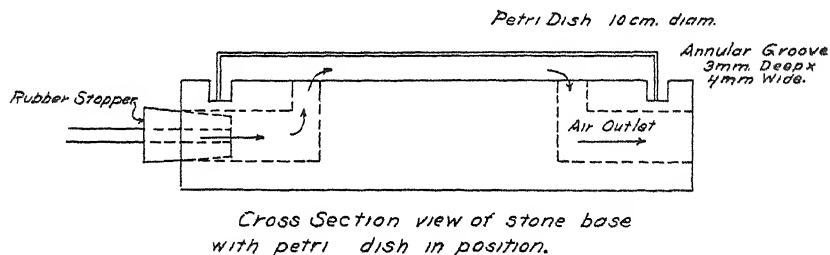
of the medium. Tilt the dish to one side, and, with a sterile
pipette, withdraw the excess of the diluting fluid. Invert the
dish into its sterile cover and allow to drain. This draining
will prevent contaminations on the stone base from spreading
upward on the plate and so obviate the necessity of sterilizing
the base. Now place the dish, edges down, into the annular
groove of the slab, which has been previously warmed to a
temperature sufficient to melt paraffine with a melting point
of say 45 degrees Centigrade. The plate may be sealed to the
stone by flowing melted paraffine into the groove either before
or after the plate is put into position in the groove. The stone
is then allowed to cool until the paraffine is thoroughly con-
gealed. There are now, between the stone and the surface of
the medium, only about 20 cc. of air to be replaced by the inert

gas, and by leading the gas from a suitable generator in through one of the rubber stoppers, (allowing the air to escape by way of the opposite loosely-stoppered opening) the oxygen pressure inside the inclosed space may be reduced to less than 1 mm. by the use of only about 200 cc. of the gas. The holes are then securely closed and the plates are ready for incubation.

Oxygen- and CO_2-free air has been secured by the following method: Connect in series one or more of the sealed Petri dishes with two wash bottles containing a 5 per cent pyrogallic acid 10 per cent caustic soda solution. Force air slowly through this train and allow the displaced air to escape by way of the opposite outlet of the sealed Petri dish.

When hydrogen is to be used for displacing the air, two wash bottles are required: One of $AgNO_3$ solution for traces of ASH_3, and one of lead acetate solution for H_2S.

In general, satisfactory anaerobic conditions are obtained when the volume of H_2 or N_2 allowed to pass through the sealed plates amounts to ten times the volume of air inclosed by the plates and their connections.

TESTICULAR INFUSION AGAR—A STERILIZABLE CULTURE MEDIUM FOR THE GONOCOCCUS

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INTRODUCTION

The use of testicular infusion agar suggested by Hirschfelder (1914) aroused the hope that a medium for the cultivation of the gonococcus had been found which might be sterilized by steam, thus avoiding the addition of raw albumin (ascitic fluid or blood) to agar with its uncertain sterility and frequent failure to support growth even when sterile. Unfortunately I cannot agree that by the use of his formula all difficulties in cultivating the gonococcus (at least in pure culture) are removed, as he claims. I have however, determined *some* of the factors affecting successsful cultivation of gonococci in comparatively large quantities upon a sterilizable agar containing infusion of testicle.

Vannod (1905) claimed that proper adjustment of the reaction with sodium carbonate facilitated cultivation of the gonococcus on so called ordinary media but the possible variation from the optimum is so slight that the method has not come into general use. One of Vannod's later contributions (1907) testifies to the general acceptance of the idea of the necessity of adding raw albumins.

More recently some success in improving the media has been attained by Schwarz and McNeil (1912) in this country with so called "salt free" veal agar, which is now generally used in the preparation of polyvalent antigens for the alexin fixation test.

Abroad, Lumiere and Chevrotier (1913) have advocated a mixture of beer wort and albumin sterilized in the autoclave, to which however, the addition of sterile horse serum is said to be advantageous, though not indispensable.   Upon this medium gonococcus cultures were found to be viable at remarkably low temperatures (Lumiere and Chevrotier, 1914).

Emile Weil and Noire (1913) have also suggested an agar containing whey, peptone, saccharose and urea.   I have failed in several attempts to corroborate their claim that gonococci would grow upon this medium.

I have not tried the cultivation of gonococci upon the egg broth of Besredka and Jupille (1913) nor according to the method of Ohlmacher (1915) upon Loeffler's blood serum but in several tests upon the starch agar of Vedder (1915) I have found it to be one of the most promising media.   However, the growth, while possibly less long lived upon testicular infusion agar, is so much more abundant that the use of the latter is recommended for the preparation of gonococcic vaccine.   It should prove equally valuable for the preparation of antigens to be used in the alexin fixation test but this remains to be determined.

CULTURES

My strains of gonococci came originally from clinically typical cases of urethritis and epididymitis, having been isolated upon blood agar and cultivated in some cases as long as two years on ascitic agar.   All were typical gram negative biscuit shaped diplococci, showing sparse growth upon rabbit blood or ascitic agar and failure of growth, at least in the second subculture, upon plain agar at 37°C.   These have been our criteria and while we have had a realization quickened by the work of Broughton-Alcock (1914) that we might occasionally exclude true gonococci thereby we have not hesitated to insist that our media should be tested particularly on the less saprophytic strains.   At the conclusion of each experiment therefore the purity of each culture was checked by gram stain and failure of growth upon plain agar at 37°C.

Torrey's strains "C," "K," "L," "N," "O," and "S" kindly supplied by Dr. Charles Krumwiede, Jr., of the New York City Board of Health, conform to the above requirements and like our own were found to grow abundantly upon testicular infusion agar.

The hope that opportunity might be found for making comparative tests of this medium in the isolation of gonococci from lesions has already deferred publication so long that it seems likely this will have to be left for some one more advantageously situated. Dr. H. E. Foster,[2] has however succeeded sufficiently often in cultivating the gonococcus from cases of gonorrhea to warrant making such comparisons.

EXPERIMENTAL WORK

Successful cultures were first secured with a slightly acid medium comprising aqueous infusion of beef testicle (500 grams per litre of distilled water), 2 per cent Witte's peptone, 2 per cent agar, 0.5 per cent glucose, and 0.3 per cent $NaH_2 PO_4$, nearly neutralized with $N/1$ NaOH and sterilized by intermittent steaming in the Arnold sterilizer on three successive days for 30 minutes at 100°C.

This formula differs from Hirschfelder's particularly in its sugar content, the advisability of which was shown by Elser and Huntoon (1909) and by Martin (1911). In each succeeding series of experiments a single factor was varied, the control consisting in a combination previously found successful; as the limits of variability of each factor involved were determined the preferable procedure was adopted for the following experiments.

Thus it was quickly shown that sterilization in the autoclave at 10 pounds pressure for 30 minutes is permissible. The substitution of veal for testicle infusion was found to yield a less vigorous growth. The optimum amount of testicle was found to be 500 grams per liter although fair growth resulted when the proportion was as low as 125 grams per liter of water. The

[2] Personal communication.

use of equal parts of veal extract seemed not to decrease the volume of growth but we have adhered to the use of testicle infusion alone.

AGAR

The amount of agar is important as shown in table 1. Media were made from the same testicular infusion in four lots with 1, 2, 3 and 4 per cent agar. After sterilization the slanted tubes were left in the incubator at 37°C. for three days to dry out

TABLE 1

Optimum amount of agar

AGAR %	CULTURE	16 HOURS	40 HOURS*	64 HOURS
	G2	None	Poor	Slight
1	G3	None	None	None
	G5	None	None	None
	G2	None	Fair	Fair
2	G3	None	Fair	Fair
	G5	Slight	Fair	Good
	G2	Slight	Fair	Good
3	G3	Slight	Fair	Good
	G5	Slight	Fair	Good
	G2	Slight	Fair	Fair
4	G3	Slight	None	Excellent
	G5	None	None	Good

* Patchy colonies respread on all tubes.

NOTE. In this and other tables a "slight" growth approximates that of *B. influenzae* upon blood agar; a "fair" growth corresponds to that of *B. typhi* upon plain agar; "good" to that of *B. coli*; and "excellent" to that of *Bact. pneumoneae*.

the surface of the slopes, a point shown to be necessary by ample experience. The slopes were inoculated from 48 hours ascitic agar cultures, incubated at 37°C. and observed daily.

Soft testicular infusion agar has in our hands regularly yielded less satisfactory results than that which was more firm and less moist, a fact apparently at variance with the experience of McCann (1896) working with cyst fluid agar, and Van Saun (1913) with "salt free" veal agar. Firm testicular infusion agar is moreover not only favorable for growth but facilitates

the removal of the gonococci without the admixture of solid particles of medium.

Warden (1915) has recently pointed out that one of the factors in the autolysis of gonococci is excessive moisture; it is suggested also that weak acids may inhibit autolytic disintegration. At any rate smears from testicular infusion agar cultures contain more whole cocci than those from ascitic agar, but whether the acid reaction due to fermentation of the glucose or the freedom of the media from excessive moisture, or both, may be held responsible in this case can not be stated with certainty. However, the factor of moisture in the media had to be reckoned with in all our experimental and practical work so that frequently where a clear cut result could not be obtained with fresh media there was little difficulty after a few days drying.

I have chosen 3 per cent agar as the most suitable for further use.

GLUCOSE

In the preparation of a portion of one lot of medium the usual glucose was omitted. The prepared slants were dried at 37°C. for 48 hours and afterwards at room temperature for four days. The media still appeared quite moist and the growths upon the controls where they appeared at all were patchy and unsatisfactory even on further incubation after respreading. Further drying at room temperature for ten days however led to a satisfactory result as shown in table 2, cultures being made from 24 hour ascitic agar slants and incubated at 37°C.

TABLE 2

Omission of glucose

MEDIA	CULTURE	16 HOURS	40 HOURS	60 HOURS
With added glucose, 0.5 per cent {	G1	Good	Excellent	Excellent
	G2	Good	Excellent	Excellent
	G3	Good	Excellent	Excellent
Without added glucose.......... {	G1	None	Slight	Slight
	G2	Fair	Good	Good
	G3	Fair	Fair	Good

The usual control tests failed to show contamination in any tube so that the above result demonstrates that growth is possible without added glucose but is not so good as with it.

That a modicum of carbohydrate is necessary is indicated by a lot of media made as usual with the exception of added glucose and from which only a portion[3] of the tissue carbohydrates had been removed by the growth of *B. communior*, in which no growth of gonococci could be secured. But media which had been so fermented and then re-inforced by addition of 1 to 2 per cent glucose yielded very excellent growth showing that inhibition in the fermented media could scarcely have been due to the accumulation of metabolic wastes from *B. communior*. Thus it was shown that for these strains the order of preference for added glucose content in media previously fermented by *B. communior* is 1 per cent, 2 per cent, and 3 per cent. Since, however, I have found no advantage in a preliminary fermentation the addition of 0.5 per cent glucose to unfermented media has been retained.

PHOSPHATES

The use of unsaturated phosphates in culture media for bacteria was recommended by Henderson and Webster (1907) for their stabilizing effect upon the reaction, and a medium of this sort plus human serum was advocated by Martin (1911) for the cultivation of the gonococcus.

My experiments upon the *necessity* of *added* phosphate have been inconclusive; at times excellent growth has been secured without its addition. In three separate double lots of testicular infusion agar made with and without the addition of inorganic phosphate the advantage has been in favor of that containing it. I have made no effort to determine the optimum amount or to attempt the cultivation of gonococcus in phosphate free media.

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[3] That the tissue sugar was not completely eliminated was proven by further gas production in deep tubes of the supposedly sugar free testicular infusion agar by *B. communior*.

REACTION

The inclusion of 0.3 per cent NaH_2PO_4 permits a considerable variation in the amount of sodium hydroxide added to reduce the titrable acidity. The results of a typical controlled experiment upon this point are shown in table 3. I might mention here having previously encountered some difficulty in the addition of

TABLE 3

Range of reaction of testicular infusion agar

CUBIC CEN-TIMETERS N/1 NaOH ADDED IN 110 cc.	TITRE		CULTURE	16 HOURS	40 HOURS	64 HOURS
	Hypothetical end point*	Actual end point				
0	+6.0	-6.0	G1	None	None	None
			G3	None	None	None
			G5	None	None	None
2	+4.0	+4.2	G1	None	None	Slight
			G3	Slight	Fair	Good
			G5	Slight	Fair	Good
4	+2.0	+3.2	G1	Fair	Excellent	Excellent
			G3	Fair	Excellent	Excellent
			G5	Good	Excellent	Excellent
6	0	+1.7	G1	Good	Excellent	Excellent
			G3	Fair	Excellent	Excellent
			G5	Good	Excellent	Excellent
2	-2.0	?†	G1'	Slight	Good	Excellent
			G3	Slight	Good	Excellent
			G5	Fair	Good	Excellent
10	-4.0	Alkaline†	G1	None	None	None
			G3	None	None	None
			G5	None	None	None

*Assuming no unsaturated compounds.

† Media darkened by caramelization—end point uncertain.

more than sufficient alkali to saturate the phosphate, the glucose being thereby caramelized on heating with resultant inhibition of growth of the gonococcus.

A liter of medium was prepared with the usual testicular infusion, 2 per cent peptone, 3 per cent agar, 0.5 per cent glucose and 0.3 per cent Na H_2PO_4. Before neutralization 5 cc. titrated hot with phenolphthalein required 6 cc. N/20 NaOH to show

color. Six lots of 100 cc. each were separated and to each was added the amount of N/1 NaOH shown in table 3, and the total volume of each lot was then equalized at 110 cc. by addition of distilled water. The various media were tubed, sterilized in the autoclave at 10 pounds for 30 minutes, slanted, and dried at 37° C. for six days. Inoculation was made from 24 hour testicular infusion agar cultures; incubation was at 37°C. and the usual control tests confirmed the purity of the growth observed.

It may be seen that "excellent" results may be expected between the limits of actual titre to phenolphthalein from below +1.7 to +3.2 Normal acidity. There will be found little if any difficulty in the reaction when adjustment is made by addition of N/1 NaOH as if the acidity were to be reduced to a titre of zero. It will still be found sufficiently acid, thanks to the phosphate, to obviate the difficulty of caramelization.

PEPTONE

The recent scarcity of Witte's peptone has necessitated experiments upon the substitution of an American product. These have shown "Difco" peptone of the American Digestive Ferments Co., Detroit, Michigan, to be equal to Witte's for this purpose.

VIABILITY

In contrast with the experience of Vedder (1915) with starch agar, prolonged viability cannot be claimed for cultures of gonococci upon testicular infusion agar. It was found that daily transfer of several strains for two weeks was eminently successful; planting every other day failed to keep some of the strains alive for more than four transfers and in all of these there was evidence of deterioration.

Inoculating testicular infusion agar as well as ascitic agar, starch agar, "salt free" veal agar or blood agar from another medium we have often found it necessary to coax the growth by repeated transfers and especially by respreading, before the maximum crop could be obtained. For this reason much emphasis should be placed upon the importance of personal experi-

ence in handling gonococcus cultures. I am pleased to thank Miss Vera Bennett who has prepared much of the culture media for me and Miss Lettie Watkins who has assisted in keeping the stock cultures alive and planting the experimental media.

SUMMARY

The formula now followed in preparing testicular infusion agar for the growth of the gonococcus in preparing suitable vaccines.

1. Mix 500 grams ground beef testicle from which the tunica vaginalis has first been stripped, with 1000 cc. distilled water.

2. Soak overnight at room temperature.

3. Heat to 50°C. Keep warm for one hour by placing in the incubator at 37°C.

4. Boil, strain, and restore to 1000 cc. with distilled water. If in excess do not reduce by boiling since overheating is injurious.

5. Add 2 per cent peptone (Witte's or Difco), 3 per cent agar chopped fine, 0.5 per cent glucose, 0.3 per cent NaH_2PO_4.

6. Soak at least one hour to soften the agar.

7. Melt in the autoclave at 10 pounds pressure for 30 minutes.

8. Titrate with phenolphthalein and add N/1 NaOH sufficient to neutralize if no unsaturated compounds were present.

9. Check the titre by repetition. 5 cc. should require from 1.0 to 2.0 cc. N/20 NaOH to display color hot.

10. Tube and sterilize in the autoclave at 10 pounds for 30 minutes.

11. Slant or pour into plates.

This medium may be melted for plating, etc., but the less heating the better. Filtration for the purpose of removing the distinct turbidity of the medium also seems to be a disadvantage.

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BOOK REVIEW

Der Erreger der Maul—und Klauenseuche. By DR. HEINRICH STAUF-
FACHER. Wilhelm Engelmann, Leipzig, 1915. 57 pages, 29 text
figures and 2 plates. M 2.80.

The cause of hoof and mouth disease of cattle has been variously
assigned, usually on *a priori* grounds, to bacteria, protozoa, and to
ultra-microscopic organisms, but no one except the author of the present
work has succeeded in satisfying the necessary postulates for the de-
termination of a disease-causing parasite. Dr. Stauffacher of Frauen-
feld, Switzerland, has found definite bodies in the blood and diseased
tissues of every animal with the disease examined; he has grown these
bodies in the condensation water of blood agar culture media and has
observed many developmental stages; he has inoculated normal cattle
with the organisms from the artificial cultures and produced the dis-
ease in such previously unaffected animals, and he has recovered the
organisms from the diseased tissues of the inoculated animal.

Students of hoof and mouth disease have been bull-dozed by author-
ity into the view that the cause must be ultra-microscopic, first, be-
cause no trace of organisms can be found in diseased tissues treated
and stained by the ordinary technical methods, and second, because
the virus passes through the ordinary filters. When no less an authority
than Löffler implies that it is a waste of time to look for the organisms
of hoof and mouth disease with a microscope, it is to be expected that
only those investigators who have authority-proof minds will under-
take the task. Such men do not forget the history of *Treponema
pallidum.* Nor is the fact that the virus of hoof and mouth disease
will pass through a filter, necessary evidence of ultra-microscopic size;
trypanosomes, to say nothing of spirochaetes, pass through ordinary
bacteria filters.

Stauffacher seems to have such an authority-proof mind; he argued
that the organisms must be in the infected tissues, and that the fact
of their not taking the ordinary stains is no guarantee that they may
not stain with altered methods. So after vainly trying to stain sections
with haematoxylin, fuchsin-methylen-blue, and other anilin combina-
tions, finding no characteristic chromatin reaction in the infected cells,
either in the nuclei or cytoplasm, he substituted acid fuchsin for the
ordinary fuchsin and found, as Zschokke had found before, that in-
fected tissues take up more acid fuchsin than do normal tissues. Even
with this modification however, he was unable to get any trace of
basichromatin material either in the nuclei or in the cell bodies. Finally,
after "months of study of thousands of sections" he discovered the im-
portant secret of treatment, after which the nuclei again took up the

characteristic methylen-blue stain, while thousands of minute bodies in and around the cells, and not seen before, were revealed. The method used was very simple: the sections were first stained for from two to six hours in dilute aqueous acid fuchsin (0.2 per cent), rinsed in distilled water and then stained for from six to ten hours in Ehrlich's fuchsin-methylen-blue mixture, then rinsed thoroughly in distilled water after which they were left in absolute alcohol until no more color came out, and were cleared in xylol and mounted in balsam.

The important part of Stauffacher's work centers in the cell inclusions and the similar bodies found in the lymph spaces and in the blood of infected animals. These are minute polymorphic structures with an average length of one micron (1 μ), and either spherical, ellipsoidal, crescentic, chain-form, comma-form, or ring-form in shape. They were found in all of the infected animals (26) examined, and were never found in similar tissues of normal, healthy animals. The same bodies were found in the freshly-drawn blood, both free in the plasma and within the red blood corpuscles of infected animals, a fact which practically excludes the possibility that they are products of nuclear and cellular degeneration brought about by the disease.

Blood was drawn from the jugular vein of an infected animal under sterile conditions, and a few drops were added to sterile tubes of blood agar prepared according to Nicolle's formula for growing *Leishmania*. Vesicles on the tongues of infected cows were flooded with sterilized distilled water which was then withdrawn and placed in similar agar tubes, one cubic centimeter to each tube. On the fourth day afterwards, the tubes containing the vesicular lymph had a decidedly milky appearance. A drop of this, examined under the microscope, showed myriads of actively moving organisms. The tubes with the venous blood showed the same picture somewhat later. Two distinct types of organisms were observed; one, shorter and thicker, had the characteristic appearance of a flagellated protozoon, with a lancet-formed body which becomes sharply attenuated and drawn out into a long flagellum. The average length of these individuals was 45 μ of which the body comprised from 20 to 25 μ with a diameter of about 3 μ. The second type was much longer and more thread-like, vith maximum dimensions of 120 μ by 1 μ. Granules within the bodies of these two types were regarded as blepharoplast, nucleus and chromidia; the bodies themselves were not metabolic, nor was there any evidence of mouth or vacuoles. Reproduction by longitudinal division was common to all types. In addition to reproduction by division, another method analogous to spore-formation was described; in this the body becomes thickly strewn with chromidia, each of which becomes the minute nucleus of an excessively small spherical structure, similar to some of the intra-cellular stages, and which might well be able to pass a filter.

Finally Stauffacher inoculated two normal cows with the uncontaminated agar culture material. The first experiment was interrupted by the mobilization of the Swiss army in August, 1914, and we are not told what became of the animal. The second experiment was successful,

the animal developing symptoms of the disease on the fourth day after subcutaneous injection of culture material. From this animal the contents of a fresh vesicle was p'aced in an agar tube, and in two days the fluid was swarming with the same kinds of organisms that had been introduced from the earlier culture tube.

From the standpoint of protozoology these results are entirely consistent with the established facts of the life histories of other cell-invading flagellated parasites. It is regrettable that the technique was such that no definite conclusions can be drawn in regard to the finer structures of the parasite. All of the tissues and smears were fixed in 70 per cent alcohol which is far from satisfactory for the interpretation of delicate structural details. Observations on the living organisms leave no doubt of the flagellum, but we do doubt a statement to the effect that this flagellum "is not a flagellum in the usual sense of the word, but rather a flagellum-like appendage to the cell." A better fixation and more careful staining would probably reveal the nucleus and blepharoplast and the insertion of the flagellum base in or near the blepharoplast. The polymorphic structure of the organisms is not an uncommon feature of allied forms of protozoa; short and stumpy types mingled with long thread-like forms are characteristic of cultural stages of *Crithidia, Leptomonas, Trypanosoma* and *Leishmania,* and similar differences in form are met with in the normal hosts.

The intra-cellular bodies with their varied forms, which, however, are reducible to one general type, are strikingly suggestive of *Leishmania donovani* of kala azar. But here again, the finer structures must remain unknown until a better method of fixation is employed.

On the whole, we are inclined to accept Stauffacher's interpretation of the organism which he names *Aphthomonas infestans,* as belonging to the group of simple flagellated protozoa (Monadida) and closely related to the genus *Leishmania.* If this work is confirmed on material fixed with better methods, we should naturally expect the next step to be the discovery of the transmitting agent in some form of tick or biting fly.

GARY N. CALKINS.

Columbia University,
March, 1916.

ABSTRACTS OF AMERICAN BACTERIOLOGICAL LITERATURE

ANIMAL PATHOLOGY

The Bacillus enteritidis as the Cause of Infectious Diarrhea in Calves.
K. F. MEYER, J. TRAUM, C. L. ROADHOUSE. (Jour. Am. Vet. Med.
Assoc., 1916, **49**, 17–35.)

In the course of an experiment on feeding calves at the Agricultural
Experiment Station of the University of California, infectious diarrhea
or scours occurred in severe form. The etiological agent was deter-
mined to be of the *paracolon* type by all the identity reactions and sero-
logical tests; and was regarded as identical with *B. enteritidis* (Gärtner).
Bacteriological findings were confirmed by feeding experiments with
two calves, of which one succumbed and one recovered.—A. R. W.

A Filterable Organism Isolated from the Tissues of Cholera Hogs. D. J.
HEALY and E. J. GOTT. (Jour. Infect. Diseases, 1916, **18**, 124–128,
1 pl.)

In the course of a previous attempt to isolate a filterable virus from
the mesenteric glands of virus hogs, the technique involved the filtra-
tion of the glands immediately after grinding. In the present investi-
gation the gland tissue after grinding was suspended in 1 per cent glu-
cose neutral beef broth at 4°C. for five days, then put through a tested
Chamberland-Pasteur "F." The filtrate was divided between two
flasks, one of which was placed in a Novy jar, the other sealed, and both
incubated at 4°C. While the anaerobic preparation showed no growth
after thirteen days, the other flask showed a distinct growth after four
days. "This growth appeared as a fine sediment in the bottom of the
flask." Upon agitation it "ascended through the fluid in the shape of a
small cloud. . . ." This "filterable organism" grew best at 37°C.
but also at 20°C. and at 4°C. Hanging drop preparations revealed
clumps of a non-motile organism surrounded by a gelatinous material.
Satisfactory stained preparations were obtained by the Giemsa method.
In such preparations the organism appeared as a coccus or a small
bacillus 0.2 to 0.3μ in diameter. Subcultures were not obtained. In
tests with immune serum complement fixation was obtained with the
culture fluid in which the organism had been grown.—P. B. H.

A Report Upon an Outbreak of Fowl Typhoid. WALTER J. TAYLOR.
(Jour. Am. Vet. Med. Assoc., 1916, **49**, 35–47.)

The writer encountered in California an outbreak of the disease
first described by Moore in 1895, as infectious leukemia, and by Daw-

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son in 1898, and studied under the name, fowl typhoid, by Curtice in 1902.

His observations led Taylor to the following conclusions:

1. Fowl typhoid is a specific disease of fowls caused by *Bacterium sanguinarium* occurring sporadically and causing heavy losses among affected flocks; which unless properly investigated may easily be mistaken for fowl cholera because of its high mortality.

2. The specific morbid conditions consist of an enlarged liver containing necrotic areas, an enlarged spleen and a general anemic condition of the serous and mucous membranes together with a marked increase in leucocytes and a corresponding decrease of the red cell content of the blood.

3. The increase in leucocytes seems to be confined to the polymorphonuclear variety.

4. Fat, well conditioned, adult fowls are more susceptible than young, nearly mature growing birds.

5. Birds may contract the disease by the ingestion of pure cultures of *Bacterium sanguinarium*.

6. Birds fed upon the offal of other birds dead of this disease show a mild non-fatal form of the disease tending to recovery.

7. There is evidence that recovery from this mild form produces more or less of an immunity. Further investigation upon this point is needed.

8. The power of some of the red corpuscles of the affected fowls to take the violet stain, when the blood is diluted in Toisson's fluid is especially noticeable in this disease.

9. While the lesions produced in fowls which are infected with *Bacterium sanguinarium* resemble in many respects those produced by *Bacterium pullorum*, and although there is a still closer resemblance in the biological characters of the two organisms, there is enough difference to warrant the conclusion that they are distinctly different diseases.

<div style="text-align: right">A. R. W.</div>

The Value of Virulent Salt Solution in the Production of Anti-Hog-Cholera Serum by the Intravenous Method. ROBERT GRAHAM and L. R. HIMMELBERGER. (Jour. Infect. Diseases, 1916, **18**, 118–123.)

Craig and Robbins have each shown that salt solution rendered virulent by remaining for some time in the peritoneal cavity of virus pigs can be used advantageously by subcutaneous inoculation to produce potent antisera in immune hogs. The present authors attempted to apply this method modified by the use of intravenous inoculation. A virulent salt solution was obtained by injecting into the intraperitoneal cavity 25 cc. of a 0.9 per cent solution per pound weight of hog. The salt solution, recovered at time of killing five hours later, represented 40 to 70 per cent of the original volume. The volume of blood obtained at the same time was found to have increased on the average by 10 to 20 per cent. Salt virus and blood virus, mixed in the proportions of 1:1 or 3:1, and inoculated either subcutaneously or

intravenously at the rate of 7 cc. per pound of body weight into immunes gave satisfactory results in the production of potent antisera when these sera were tested on susceptible shoats infected with 2 cc. of virus. The conclusions are reached that an immune serum may be produced and used more economically when mixed salt virus and blood virus are employed and injected by the intravenous method, since the total volume of available virus solution is raised by this procedure from 75–80 per cent.—P. B. H.

Experiments to Determine the Relative Value of Trikresol and Carbolic Acid in the Preservation of Hog Cholera Serum. JOHN REICHEL. (Mulford's Vet. Bul., 1916, **7**, 61–64.)

Carbolic acid is generally used as the preservative of hog cholera serum, even though comparatively little is known of its value as a germicide in such a product as hog cholera serum in the form of defibrinated blood as originally prepared by Dorset, McBryde and Niles. That carbolic acid itself has little or no effect on the potency of the product is conclusively established.

Whether trikresol is equally harmless and as effective as a preservative remains to be shown.

Hog cholera serum as generally prepared is not sterile. The blood as drawn from the serum-producing animal is invariably contaminated and subsequent handling in defibrination allows for additional contamination, up to the time the preservative is added, varying in degree with the care exercised in its preparation. Even though it is possible to obtain sterile blood from a serum-producing hog, this can only be said of experimental trials and in producing hog cholera serum defibrinated blood, in a practical way, the question of sterility must be entirely sacrificed.

Carbolic acid will not sterilize this contaminated product and the question naturally arises "does it hold the organisms in check whether present in large or small numbers?"

The writer observes that:

1. Carbolic acid must be used in less than 0.75 per cent to avoid changes in the physical appearance of hog cholera serum defibrinated blood.

2. Trikresol must be used in less than 0.6 per cent for the same reason.

3. Carbolic acid added in amounts up to 0.75 per cent to lightly or heavily contaminated defibrinated blood first caused a decrease in the number of bacteria followed by an increase exceeding the first bacterial count.

4. Trikresol with a carbolic acid coefficient of 2.87 added in amounts up to 0.6 per cent was also followed by a decrease, then an increase practically equal to that which occurred in the carbolized samples.

5. All of the controls, without any preservation, showed an increase in the bacterial count from the time the samples were first set aside along with those to which a preservative had been added. This

increase was followed by a noticeable decrease, and the last count was on an average lower in the control samples than those treated. That putrefactive changes took place was appreciated by the odor and liquefied appearance of the product.

6. No odor or putrefactive changes were observed in the carbolized and trikesolized samples. Both preservatives served well in this respect, but this alone must not be accepted as proof of the value of either preservative for defibrinated blood.

7. From the limited number of examinations made in these experiments as to the types of bacteria which survive and then flourish in the carbolized and trikresolized samples, it can be said that the types were not limited to the spore-forming bacteria alone, as organisms of the colon type, staphylococci and streptococci were found as long as the samples were kept.

8. Contaminated hog cholera serum defibrinated blood cannot be sterilized by the addition of carbolic acid and trikresol in practical amounts, and the numbers of bacteria are not kept in check by the preservative.

9. No evidence is brought forth here to show that carbolic acid or trikresol would not serve well as preservatives when added to a sterile product.

10. Hog cholera serum must be prepared in a sterile manner or sterilized by one means or another to enable carbolic acid or trikresol to serve as a satisfactory preservative.

11. The physical nature of hog cholera serum defibrinated blood, probably has much to do with the limitations of carbolic acid and trikresol as preservatives, and it is highly probable that both would prove more effective if the insoluble, inert material, fibrin cellular débris, etc., were eliminated from hog cholera serum. These inert materials undoubtedly exert a large influence in the complications following the use of the product, and for this reason alone should not be allowed to remain in hog cholera serum on the market.—A. R. W.

BACTERIOLOGY OF AIR AND DUST

Recovery of Streptococcus viridans from New York Street Dust. W. C. THRO. (New York Med. Jour., 1916, **103**, 444–445.)

Cultures made from New York street dust, collected at the level of the second floor revealed the presence of *Bacillus fluorescens* once; a member of the colon group, probably paracolon, once; chromogenic Gram positive cocci several times; and *Streptococcus viridans* seven times. The strains of *Streptococcus viridans* were tested for their fermentative properties and their pathogenicity for mice and rats.

M. W. C.

BACTERIOLOGY OF FOODS

Food Poisoning by the Bacillus Paratyphosus B. HARRY S. BERN-
STEIN and EZRA S. FISH. (Journ. A. M. A., 1916, **66**, 167.)

Report is made of an epidemic of food poisoning at Westerly, R. I.
Sixty persons were made seriously ill, four of whom died. Symptoms
of gastric disorder occurred within $4\frac{1}{2}$ to 19 hours after eating pie
obtained from a local restaurant.

In an analysis of the ingredients of the pies an organism was isolated
possessing the morphologic, serologic and cultural characteristics of
Bacillus paratyphosus B.—G. H. S.

Indol in Cheese. V. E. NELSON. (J. Biol. Chem., 1916, **24**, 533.)

N. determined presence of putrefactive products, indol, skatol and
phenol in different cheeses. Indol present in limburger and camem-
bert, phenol only in former. Skatol absent in both. Cheddar, swiss
and roquefort are free from these substances. Lactic and bulgarian
bacilli and a liquefying coccus were grown in a medium containing
tryptophan, 4 per cent lactose and salts. First two failed to pro-
duce indol; last produced small amounts. N. believes that medium
was probably unfavorable for growth of former. He apparently
disregards the sparing effect of lactose.—I. J. K.

BACTERIOLOGY OF SOILS

Relation of Green Manures to the Failure of Certain Seedlings. E. B.
FRED. (J. Agr. Res., 1916, **5**, 1161–1176.)

It has been observed that germination of seeds is poor on soil to
which green manures have been recently added. The writer reports
the result of an investigation to show whether this may not be due to
micro-organisms (bacteria or fungi) that develop in large numbers in
decomposing green· manures. The indications point to fungi as the
harmful agents. Some fungi have been isolated from decomposing
green clover that are very destructive to seedlings. Oily seeds are
easily damaged by the fungi, but starchy seeds are very resistant.
Damage to seeds by green manures is generally confined to the first
two weeks after their addition to the soil. Small applications of
calcium carbonate seem to increase the injury. In all cases where
the germination is slow, a high percentage of the seedlings prove to
be diseased.—H. J. C.

Relation of Carbon Bisulphid to Soil Organisms and Plant Growth.
E. B. FRED. (J. Agr. Res., 1916, **6**, 1–19.)

It has been shown in the past that if soil is treated with carbon bi-
sulphid there is an initial decrease in the number of micro-organisms
which is followed by a large increase in their numbers and in the amount
of nitrate and ammonia produced, as well as by increased plant growth.
The writer confirms these conclusions.

One theory commonly held to explain these results is that carbon bisulphid kills certain of the micro-organisms, thus allowing other kinds to increase to abnormal numbers and to supply the plants with an unusual amount of available nutrient matter. Another theory, held by A. Koch, is that the carbon bisulphid in the small quantities used is a direct stimulant to bacteria and to higher plants.

The writer's data tend to support Koch's theory. Carbon bisulphid does not act alike in all soils or toward all crops. There is an increased growth of plants in sand culture (pure silica sand with nutrient solution added) as well as in soil. Of all the crops investigated, mustard (which contains sulphur) receives the most striking benefit from the treatment (except in acid soils). Next to mustard comes rape, then red clover, then buckwheat, then oats, while corn is scarcely benefited if at all.—H. J. C.

BACTERIOLOGY OF THE MOUTH

Deep Seated Alveolar Infections. M. L. RHEIN. (Surg., Gynecol., and Obstet., 1916, **22**, 33–37.)

One case of arthritis is mentioned. *Streptococcus viridans* was isolated from the pulp of an apparently sound tooth. Patient made good recovery.—C. P. B.

The Dental Aspect of the Relation of Endamoeba to Pyorrhea Alveolaris. W. A. PRICE, D.D.S., M.S. (Surg., Gynecol., and Obstet., 1916, **22**, 37–43.)

The author's subject was really Cinematographic Film studies showing the movements of mouth organisms, including endamoeba. This method should lend itself to studies of most bacterial forms.

C. P. B.

On the Cultivation of Entameba buccalis. WM. B. WHERRY and WADE W. OLIVER. (Lancet Clinic, 1916, **115**, 295.)

Wherry and Oliver found that the *Entameba buccalis* grew best on "Martin's pleuritic" medium, made up with basic sodium phosphate, and pleuritic fluid in the proportion of about two of the fluid to three of the agar. The tubes were slanted and allowed to remain in the ice box, so that the water of syneresis could collect. The *Entameba* and bacteria from the margin of the tooth were introduced into the water of syneresis and incubated at 35° to 37°C. There was a profuse growth at the end of 48 hours; staining with Mallory's ferric chloride-hematoxylin, showed characteristic nuclear structure. The authors hope to be able to throw some light on the life history of this organism and to make tests of its pathogenicity.—O. B.

The Relation of Amoebiasis to Pyorrhea Alveolaris. A. H. SANFORD, M.D. and GORDON B. NEW, M.D. (Surg., Gynecol. and Obstet., 1916, **22**, 27–33.)

The authors studied 327 patients, dividing them into 5 groups. Material from pyorrheal pockets, also from sto ls was examined. Of

73 patients with *Entameba histolytica* present in the stools only 31 also had *Entameba buccalis* in the mouth while in 254 cases in which parasites were absent from the stools, 150 had *Entameba buccalis* present.

Dogs were inoculated about the teeth with pus containing amoebae from pyorrheal pockets. In subsequent examination no signs of pyorrhea were present and amoebae could not be found.

Kittens given intracaecal inoculations of *Amoeba histolytica* developed typical dysentery while those inoculated with *Amoeba buccalis* showed no signs of dysentery.—C. P. B.

The Dental Path: Its Importance as an Avenue to Infection. T. B. HARTZELL, M. D. and A. T. HENRICI, M.D. (Surg., Gynecol., and Obstet., 1916, **22**, 18–27.)

In a series of acute dental abscesses staphylococci were the active organisms, in 250 cases of chronic abscess *Streptococcus viridans* was the predominating organism.

Heart lesions were produced in rabbits, injected with cultures of *Streptococcus viridans* and other streptococci obtained from pyorrheal pockets and saliva, while some strains seemed to have a predilection for the kidneys.

In one case death was due to the fusiform bacillus, the organism being isolated from the blood. Another died of pneumonia, the primary infection with the pneumococcus occurring in a bicuspid tooth.

Comment. Many authors now classify cocci producing greenish color on blood agar, not pneumococci, as *Streptococcus viridans*. It would seem desirable that the authors should determine whether the organism is the classic *Streptococcus viridans*, that is a pin head grayish rough irregular colony, which gradually becomes brownish producing a greenish color, in the blood agar surrounding the colony and grows in very long chains in bouillon, the elements of which are diplococci; or simply belongs to the group which produces the green color when grown on blood agar.—C. P. B.

BACTERIOLOGY OF WATER AND SEWAGE

The Electrical Treatment of Water. T. A. STARKEY. (American Medicine, 1916, **11**, 181.)

S. passed currents of electricity through water containing *B. coli* and *B. prodigiosus*. He found that the current as such had very little effect on the germs. He used both direct and alternating currents and varied the amperage from 0.1 to 2.0 and the voltage from 90 to 40,000.

He found that the gases produced by the electrolysis did have some germicidal power. In one case the count was reduced from 34,000 to 2,000. In no case was the water made entirely sterile. S. does not state whether there was any salt in the water from which hypochlorite might have been made.

In another series he tried to test the sterilizing value of any metallic

salts that separated from the plates (electrodes) and obtained complete sterility in a few cases. He does not, however, state what material composed the plates or what salts passed out into the water. The report is so incomplete as to be of little value.—E. C. L. M.

CLASSIFICATION OF BACTERIA

Grouping of Meningococcus Strains by Means of Complement Fixation. Miriam P. Olmstead. (Proc. N. Y. Pathol. Soc., 1915, **15**, 136–143.)

Forty strains of meningococci were used but only twenty-nine were completely tested by complement fixation. Of these, fourteen fell into one group, eight into another, two cross-fixed with each other only, two failed to fix with any other strain and three acted irregularly. All strains could be clearly distinguished from the gonococcus. Culturally they were all alike. The para-meningococci are regarded as constituting a special strain among meningococci, not, however, a wholly homogeneous one.—W. J. M.

DISINFECTION

The Standardization of Disinfectants. J. T. Ainslie Walker. (New York Med. Jour., 1916, **103**, 500–505.)

A critical comparison of the Hygienic Laboratory and Rideal-Walker tests, leading to the conclusion that the Rideal-Walker test is superior to the Hygienic Laboratory method in every one of the points discussed.
M. W. C.

Soap. G. K. Dickinson. (Medical Record, 1916, **89**, 556–558.)

Work done up to the present time upon the antiseptic and bactericidal action of soap has not been sufficiently uniform in conditions or methods to draw from it hard and fast conclusions.

In general, it may be said that all soaps possess some disinfectant power by virtue of the alkaline reaction alone. All bacteria exposed to soap solutions are not, however, killed in the same time, and a considerable interval is necessary before any practical disinfection can occur. Most so-called disinfectant soaps have no value beyond that of ordinary soaps. A combination of soap and biniodide of mercury is a useful disinfectant, but it does not produce complete sterility
M. W. C.

IMMUNOLOGY

Therapy as Related to the Immunology of Tuberculosis. E. R. Baldwin. (New York Med. Jour., 1916, **103**, 532–534.)

A discussion of the relation of immunology to the methods of treatment now used in tuberculosis.—M. W. C.

Vaccines of Favus and Ringworm. C. H. LAVINDER. (Journ. A. M. A. 1916, 66, 945–946.)

A method is given for the preparation of vaccines from the fungi which cause the above infections.—G. H. S.

Treatment of Cases of Epidemic Meningitis. J. B. NEAL. (Journ. A. M. A., 1916, 66, 862–864.)

The author places emphasis upon the value of continued injections of antimeningitis serum even though the patient shows improvement.

Autogenous vaccines have been employed in cases which tended to become chronic.—G. H. S.

The Effect of Moderately High Atmospheric Temperatures upon the Formation of Hemolysins. C.-E. A. WINSLOW, JAMES ALEXANDER MILLER, and W. C. NOBLE. (Proc. Soc. Exp. Biol. and Med., 1916, 13, 93–98.)

Rabbits kept at a temperature of 29° to 32°C. were compared with control rabbits kept at 18° to 21°C. by injecting them with washed sheep erythrocytes and subsequently testing the specific hemolytic activity of the serum. Hemolysin formation was relatively delayed in the animals kept at the higher temperature, but at the end of four weeks the titre was as high in these as in the controls. Considerable individual variation within the groups was observed.—W. J. M.

An Allergic Skin Reaction to Diphtheria Bacilli. J. A. KOLMER. Proc. Soc. Exp. Biol. and Med., 1916, 13, 89–91.

Diphtherin was prepared by suspending washed diphtheria bacilli in salt solution and sterilizing the suspension at 60°C. for an hour. Each cubic centimeter contained approximately two billion bacilli. An intracutaneous injection of 0.1 cc. of diphtherin was used for the test, and the usual Schick test was also made at the same time. The two tests agreed in 63 per cent of the patients tested. The diphtherin test is regarded as an index of bacteriolytic immunity whereas the Schick test is an index of antitoxic immunity.—W. J. M.

The Influence of Typhoid Bacilli on the Antibodies of Normal and Immune Rabbits. C. G. BULL, Journ. Exp. Med., 1916, 23, 419–429.

The subcutaneous, intraperitoneal, or intravenous inoculation of cultures of typhoid bacilli did not cause, as far as could be determined, a decrease in the antibody content of the blood serum of the rabbit. On the other hand, the intravenous inoculation of typhoid bacilli causes a rapid mobilization of antibodies, thus increasing their concentration in the blood, to be followed somewhat later, by the production of so-called acquired antibodies. No such condition as the negative phase of Wright was discovered, although it was especially looked for in the experiments.—G. B. W.

A Method for the Rapid Preparation of Anti-Meningitis Serum. H. L. AMOSS and MARTHA WOLLSTEIN. (Jour. Exp. Med., 1916, **23**, 403–417.)

The method described by the authors consists of three successive intravenous inoculations of many strains of living meningococci and parameningococci repeated at stated intervals. Anaphylactic dangers are obviated by preliminary desensitizing injections and the doses are adjusted according to the febrile reaction. The great advantage of the method is that a polyvalent serum of high titer can be produced in 8 to 12 weeks instead of in the 10 months required by the subcutaneous method. This investigation offers a promising suggestion for the production of other immune sera.—G. B. W.

Variations in the Strength of Positive Wassermann Reactions in Cases of Untreated Syphilis. D. A. HALLER. (Journ. A. M. A., 1916, **66**, 882–884.)

From an examination of over 6000 Wassermann reactions in which but one antigen was used it appears that amboceptor is the only constituent of the hemolytic system which is a constant. All other factors may vary and will account for the difference in daily determinations of the fixing unit of a positive serum.

The titer of sera from cases of untreated syphilis remains the same from day to day or from month to month.

The administration of mercury may quickly change a positive to a negative reaction, and upon stopping the treatment the reaction may as quickly become positive again.—G. H. S.

Continuous Transfusion; The Production of Immunity. An Experimental Study. A. KAHN. (Medical Record, 1916, **89**, 553–556.)

Dogs were infected by opening the peritoneal cavity and inserting a small quantity of dust, gauze saturated with pus, or pure pus. After an interval, varying from 1 to 5 days, the donor and the infected dog were prepared for transfusion and a continuous flow of blood from one animal to the other was allowed to occur from $\frac{1}{2}$ to 3 hours.

In dogs that were not transfused following infection, death occurred in 24 to 48 hours; in transfused dogs death was deferred from 3 to 4 days, or absolute recovery took place.

Transfusion raises the vital resistance. Whether or not immunity is produced is not known.—M. W. C.

Possible Reasons for Lack of Protection after Antityphoid Vaccination. HENRY J. NICHOLS. (The Military Surgeon, 1916, **38**, 263–268.)

Summary of article as given by author is:

1. False failures in immunization may be due in some cases to the difficulties of exact clinical diagnosis.

2. The uncertain duration of immunity following vaccination may account for some true failures. At present in the Army one revaccina-

tion of three doses is compulsory four years after the first course of three doses.

3. The kind of vaccine used may account for some failures.

    a. Whole killed vaccine.

        (1.) The strain of bacillus used may not be suitable.

        (2.) The method of preparation may be faulty.

        (3.) The vaccine may be too old.

    b. Sensitized vaccine.

        (1.) Sensitization may diminish the immunizing properties of the vaccine.

        (2.) Discarding the supernatant fluid may lessen the immunizing power of the vaccine.

4. The conditions of a soldier's life do not protect him from exposure to typhoid fever in his vicinity.

5. The Army vaccine is probably superior to some of the vaccines available for the general population.

The writer comes to the conclusion that soldiers are better protected than those who become infected after vaccination in civil life, and that this protection must be due either to better vaccination in point of numbers of doses and intervals of revaccination, or to a better vaccine. Of the two factors the vaccine is probably the most important.

                                    E. B. V.

Methods of Using Diphtheria Toxin in the Schick Test and of Controlling the Reaction. ABRAHAM ZINGHER. (American Journal of Diseases of Children, 1916, **4**, 269–277.)

The Schick reaction consists in the intracutaneous injection of one-fiftieth M.L.D. of well ripened diphtheria toxin and indicates the absence or presence of a protecting amount of antitoxin in the blood according to whether there is or is not produced a local inflammatory reaction. In some individuals there occurs a so-called pseudo-reaction which probably bears no relation to the free toxin but is the result of an anaphylactic reaction with the proteins of the diphtheria bacilli. The true and the false reactions can usually be distinguished by their appearance and time of occurrence but as a further control a super-heated (75°C.) toxin or one which has been over neutralized by the addition of two units of antitoxin to each L plus dose of toxin may be injected into the opposite arm.

The author also emphasizes the importance of careful technique in giving the injection so that it is definitely intracutaneous. The value of the test now seems well established and especially prepared vials containing undiluted toxin and with directions concerning its dilution are prepared by the New York City Board of Health and also by commercial laboratories.

Of a thousand children admitted to the Willard Parker Hospital, who gave a negative Schick reaction and who were more or less exposed to diphtheria not a single one developed the disease.

Tests on 2700 normal children in orphan asylums between the ages

of 2 and 16 years show that from 17 to 32 per cent give a positive reaction and are therefore probably susceptible to the disease.

<div align="right">R. M. T.</div>

Preliminary Notes on Skin Reactions Excited by Various Bacterial Proteins in Certain Vasomotor Disturbances of the Upper Air Passages. J. L. GOODALE. (Boston Med. and Surg. Jour., **174**, 223–226.)

G. finds that many patients who suffer from perennial vasomotor disturbances of the nasal mucous membrane give a positive anaphylactic skin reaction to extracts of certain bacteria that are commonly found in vasomotor rhinitis. Among these are *Staphylococcus albus, aureus* and *citreus, Micrococcus tetragenus* and an unidentified bacillus somewhat like Friedlaender's bacillus.—E. C. L. M.

Pollen Therapy in Pollinosis. S. OPPENHEIMER and M. J. GOTTLIEB. (Medical Record, 1916, **89**, 505–508.)

Hay fever, or pollinosis, is caused in persons having a predisposition to anaphylactic diseases, by irritation of any denuded surface of the body by proteins of pollen. One or more of a large variety of pollens may be responsible for an attack of pollinosis in a susceptible individual.

The method most frequently employed for determining which pollen or pollens are operative in a given case is the skin scarification or cutaneous method. Complement fixation tests may also be made both as an aid in diagnosis and as an indicator of immunity.

Infections, caused by streptococci, pneumococci, etc., are often complicating factors in pollinosis. In such cases an autogenous vaccine should be administered in conjunction with the specific pollen antigen.

Treatment of pollinosis proper may consist either of active immunization with pollen extract or passive immunization with the blood serum of animals that have been actively immunized with pollen extract.

The results obtained with treatment with pollen extract, in cases of spring pollinosis show 50 per cent of seasonal cures for 1913–1914, while of 32 cases treated in 1915 before the time of attack, only two had symptoms.

Of 62 cases treated for fall pollinosis 52 began treatment early enough to acquire an active immunity before the usual time of attack. Of these, 15 were free from symptoms, 25 were markedly improved, and 12 were in no way affected by the treatment. Of the 10 cases that did not begin treatment until after the onset of the attack, 4 were favorably influenced by one or two injections.

The authors caution against using "hay fever" vaccines which contain a mixture of a large number of pollen extracts, as patients should receive extracts of only those pollens which have been shown by diagnostic means to be operative. Care should be taken in the dosage of pollen extracts, as in large doses they are extremely dangerous.

<div align="right">M. W. C.</div>

Complement Fixation in Intestinal Parasitism of Dogs. JOHN A. KOL-
MER, MARY E. TRIST and GEORGE D. HEIST. (Jour. Infect. Dis-
eases, 1916, **18**, 88–105.)

The aim of the investigation was to ascertain by complement fixa-
tion tests whether the absorption of foreign substances and conse-
quent production of specific antibodies occurred in dogs infected with
intestinal parasites. For antigens were used salt solution and alcoholic
extracts of various species of *Tenia, Dipylidium, Ascaris* and *Strongylus.*
The antisheep hemolytic system was employed. Each one of 172
dog sera was tested with all of the antigens. Serum tests and feces
examinations together were made in 110 cases. The results of feces
examinations showed infections as follows: *Ascaris* (23 per cent),
Ascaris and *Trichocephalis* (20 per cent), *Tenia* (6 per cent), *Dipylidium*
(3.6 per cent), no infection (26 per cent). These results did not con-
form with the serum examinations since (1) dogs showing the ova of
certain parasites failed to react with the corresponding antigen, and
(2) positive reactions were frequently obtained with antigens of types
whose eggs were not found in the feces. The analysis of the data,
however, leads the authors to conclude that the production of anti-
bodies may occur after infestation with the common intestinal para-
sites. Such antibodies were in special evidence in tapeworm infesta-
tions, less so in round worm and only slightly in whip worm infestations.
The reactions as a whole are stated to have suggested a biologic rela-
tion between the tapeworms *Tenia serrata* and *Dipylidium caninum,*
and between *Ascaris canis* and *Strongylus gigas.* The authors state
that complement fixation tests may be of value in the diagnosis of
intestinal parasitism of man.—P. B. H.

*Studies in Non-Specific Complement Fixation: I. Non-Specific Com-
plement Fixation by Normal Rabbit Serum.* JOHN A. KOLMER and
MARY E. TRIST. (Jour. Infect. Diseases, 1916, **18**, 20–26.)

The authors direct attention to the fact that fresh active sera from
normal rabbits, in doses of 0.1 cc. show non-specific fixation with
lipoidal extracts in 5 to 15 per cent of sera tested. When the same
sera were inactivated by heating fixation occurred in 38 to 49 per cent
of sera. In the case of both active and inactivated sera the percentage
of positive reactions increased in the following order when the sub-
stances named were used as antigens: (1) alcoholic extract of heart
muscle reinforced with cholesterin, (2) alcoholic extract of syphilitic
liver, (3) extract of acetone insoluble lipoids. With bacterial anti-
gens (staphylococci, colon, typhoid) fixation occurred in some degree in
31 to 42 per cent of cases, with active sera, and in 51 to 62 per cent when
inactivated sera were used. The rabbits tested were conservative in
their reactions, 80 per cent being persistently positive or persistently
negative in successive examinations. The authors conclude by recom-
mending that "when rabbits are to be employed for experimental studies
with a view to using their sera for complement-fixation tests, their
sera should be tested one or more times before inoculation preferably

with the particular antigen to be used, and only those selected that react negatively."—P. B. H.

Studies in Non-Specific Complement Fixation: II. Non-Specific Complement Fixation by Normal Dog Serum. JOHN A. KOLMER, MARY E. TRIST and GEORGE D. HEIST. (Jour. Infect. Diseases, 1916, 18, 27–31.)

The study was undertaken to ascertain whether normal dog serum would fix or absorb complement with lipoidal and bacterial antigens as had been found to be the case with normal rabbit serum. The technique was that of the Wassermann reaction. It was found that the dog sera tested, whether active or inactivated, are capable of absorbing complement in a large percentage of cases, the greater number of positive reactions appearing in the case of bacterial antigens. When lipoidal antigens were used the order of positive reactions varied exactly as in the case of rabbit serum (*vide supra*). The best reactions with active dog serum were obtained when 0.05 cc. was used. Heating the sera at 55°C. for 30 minutes greatly increased the power for fixation for both groups of antigens, while by heating at a higher temperature the power was lessened. The authors conclude that in complement fixation tests with dog serum, "it would appear advisable to use the serum in a perfectly fresh and active condition in doses of 0.01 to 0.2 cc., after heating the serum at 62°C. instead of 55°C. for half an hour, since this removes, or greatly diminishes the tendency toward non-specific fixation of the complement."—P. B. H.

Studies in Non-Specific Complement Fixation: III. The Influence of Splenectomy and Anesthetics on the Non-Specific Complement Fixation Sometimes Shown by Normal Rabbit and Dog Sera. John A. KOLMER and RICHARD M. PEARCE. (Jour. Infect. Diseases, 1916, 18, 32–45.)

The aim of the investigation was to gain some understanding of the part played by the spleen in hemolysis, and in the increased resistance of erythrocytes after splenectomy. Pre-operative and post-operative sera were tested in both active and inactivated condition in doses of 0.1 cc. against three lipoidal extracts (*vide supra*) and two bacterial antigens (Staphylococci and *B. coli*). Ether, chloroform and nitrous oxid were employed as anesthetics. The results of the experiment showed that anesthetics, as employed, weaken or remove temporarily the power of normal rabbit and dog sera of fixing or absorbing the complement with lipoidal and bacterial antigens in a non-specific manner. "This alteration usually is not apparent at once after the administration of the anesthetic but is found after one to three days; later the serum returns to its former power of causing this non-specific fixation." Ether-administration was not found to reverse the reaction of negatively-reacting sera. Nitrous oxid oxygen had no appreciable influence on the serum reactions of normal rabbits. "Splenectomy alone probably has no influence upon the property in normal rabbit and

dog sera of fixing or absorbing complement with various non-specific lipoidal and bacterial antigens, the effect being in larger doses attributable to the anesthetic; the changes observed in dogs following splenectomy under ether were somewhat more profound than those in rabbits."

P. B. H.

Studies in Non-Specific Complement Fixation: IV. The Relation of Serum Lipoids and Proteins to Non-Specific Complement Fixation with Normal Rabbit and Dog Sera. JOHN A. KOLMER. (Jour. Infect. Diseases, 1916, **18**, 46–63.)

The aim of the present study was to determine the relation of serum lipoids to the process of non-specific complement fixation (1) by extractions of serum with lipoid solvents (ether, chloroform, etc.), and (2) by feeding and immunization experiments with various lipoids. The method employed antilytic and Wassermann tests with rabbit and dog sera, both active and inactivated (56°C. for one-half hour), before and after extraction. It was found that both serum lipoids and proteins were concerned in the antilytic and non-specific complement fixation; also, that extraction with ether or chloroform usually diminished the antilytic and complement-fixing powers of a serum, while enteral and parenteral administration of lipoids increased the antilytic and complement-fixing powers. Sera extracted with ether were rendered more antilytic, but heating an extracted serum reduced the antilytic titer compared with plain heated serum. It was further concluded "that both the globulin and albumin (filtrate) fractions of normal rabbit and dog sera possess thermostabile antilytic and complement-fixing properties . . . . The antilytic and complement-fixing substances of normal rabbit and dog serum are not dialyzable."—P. B. H.

Studies in Non-Specific Complement Fixation: V. The Effect of Heat on Normal Rabbit and Dog Sera in Relation to Antilytic and Non-Specific Complement Fixation Reactions. JOHN A. KOLMER and MARY E. TRIST. (Jour. Infect. Diseases, 1916, **18**, 64–87.)

The authors had already shown (1) the ability of normal rabbit and dog sera to yield non-specific complement fixation with various bacterial and lipoidal antigens; (2) the influence of anesthetics upon this property and (3) the relation of serum lipoids to the process. The aim of the present investigation was to study the influence of certain factors and methods for lessening its effects in complement fixation tests. The tests were conducted with lipoidal extracts and with three bacterial antigens previously mentioned (*vide supra*), the doses being the same as used in Wassermann tests. Guinea pig complement was used. The hemolysins were antisheep (rabbit), antihuman (rabbit) and antiox (rabbit). Tests for the antihemolytic properties of serum were performed by (1) incubating heated serum and complement for one hour; (2) adding the cells and two units of hemolysin; (3) re-incubating for one hour. The complement fixation tests were

conducted by (1) incubating antigen, serum and complement for one hour; (2) adding cells and two units of hemolysin; (3) re-incubating for one hour. The authors conclude from their study as follows: "(1) Non-specific complement fixation by normal rabbit and dog sera is probably due primarily to thermolabil and thermostabil antilytic (anticomplementary) substances in the sera. (2) While fresh and active rabbit and dog sera may yield non-specific complement fixation the tendency is greatly increased as a result of heating the sera. At 56°C. the changes may occur in 20 minutes or even less; at 62°C. for 30 minutes the tendency for non-specific reaction is much decreased and is entirely removed by heating serum at 70°C. for 30 minutes. Changes may occur after exposure at 45°C. for 30 minutes, but the optimal temperature is between 55° and 60°C. (3) In complement fixation tests for specific antibodies with inactivated rabbit, dog and mule sera, it is advisable to heat the sera at 62°C. for one-half hour and to use at least two units of complement or hemolysin and no more than one-quarter of the anticomplementary unit of antigen after it has been carefully titrated. (4) Complementoids and amboceptoids probably bear no relation to the process of non-specific complement fixation by rabbit and dog sera. (5) The blood corpuscles of various animals and various bacteria may absorb a portion of the antilytic substances from rabbit and dog sera, but they have much less influence on the complement fixation reactions. Digestion of fresh sera with corpuscles and bacteria not infrequently increases the anticomplementary properties of the sera. (6) Bacteriolytic amboceptors are not responsible for non-specific complement fixation by normal rabbit and dog sera. (7) Parasitic infestations of rabbits and dogs bear no relation to the antilytic and complement fixing properties of the sera. (8) Single, large doses of salvarsan are without definite influence on the reactions with rabbit serum. (9) Quantitative factors in the hemolytic system and antigen are of considerable importance in relation to these non-specific reactions. (10) If time permits, preliminary complement fixation tests should be performed with the sera of rabbits or dogs before immunization or inoculation is begun, and only those animals selected the sera of which react negatively with the antigen used."—P. B. H.

INDUSTRIAL BACTERIOLOGY

The Importance of Bacterium bulgaricus Groups in Ensilage. O. W. HUNTER and L. D. BUSHNELL. (Science, 43, 318–320.)
Various kinds of ensilage were examined at different stages of fermentation. On acidulated glucose agar only *Bacterium bulgaricus* and yeasts developed. The colonies of *B. bulgaricus* resembled *Bacterium lactis* and the authors believe that it is on this account that other investigators have overlooked them. It is concluded that the Bulgarian groups occur in sufficiently large numbers, and at a proper stage in ensilage fermentation, to play an important rôle.—C. M. H.

LABORATORY TECHNIQUE

An Eye-Shade for Use with the Microscope. E. KELLERT. (Jour. A. M. A., 1916, **66**, 1023–1024.)

A device for attachment to the draw-tube of the microscope is described. It is so designed as to prevent diffused light from entering the eye above the ocular.—G. H. S.

An Apparatus for Filling Vaccine Ampoules. R. G. Davis, U. S. Naval Med. Bulletin, 1916, **10**, 311–313.

By the use of this apparatus which is briefly described and figured, and which can be made in any laboratory it is claimed that ampoules may be filled with vaccine without loss of time or vaccine.—E. B. V.

The Use of the Sand Tube in Isolating the Bacillus typhosus. M. D. LEVY. (Journ. A. M. A., 1916, **66**, 1022–1023.)

A pipette 33 cm. long and 5 to 6 mm. in diameter is bent in a U shape. Sand is placed in one arm to a height of 10 cm. and the other arm is filled with hot bouillon. The bouillon is inoculated and the tube incubated for 18 hours.

Motile bacilli, such as *Bacillus typhosus* or occasionally *Bacillus coli*, penetrate through the sand and may be isolated from the bouillon above the sand.—G. H. S.

On a Rapid Method of Cultivating the Gonococcus. WM. B. WHERRY and WADE W. OLIVER. (Lancet Clinic, 1916, **115**, 306.)

The authors found that gonococci from the urethral pus of a boy, grew best on Martin's pleuritic agar, under partial oxygen tension.

Tubes similarly inoculated and grown aerobically, yielded no growth.

The partial oxygen tension was secured by attaching the culture tubes inoculated with the pus containing gonococci, to similar tubes inoculated with *Bacillus subtilis*. When isolated in this way the gonococci can not be subcultured aerobically, but partial tension subcultures grow promptly.—O. B.

A New Method of Separating Fungi from Protozoa and Bacteria. N. KOPELOFF, H. C. LINT and D. A. COLEMAN. (Bot. Gaz. 1916, **61**, 247–250.)

The dilution method followed by the peculiar manner of plating, makes it possible to separate fungi from bacteria and protozoa.

As the result of this separation it has been possible to eliminate fungi from experiments involving the effect of protozoa on bacterial activity, by making a sub-culture from the fungi-freed solution of bacteria and protozoa.

In view of the fact that fungi are capable of producing ammonia, their presence may introduce a factor not accounted for in measuring the effect of soil protozoa on soil bacteria.—J. T. E.

Study of the Blood with a New Stain. B. LEMCHEN. (Medical Record, 1916, **89**, 607–608.)

The stain consists of a saturated solution of benzidine in absolute alcohol. Blood smears are made on slides and placed in the stain for one-half minute. The slide is then placed in hydrogen peroxide for one-half minute, washed in water and dried on filter paper.

In studying blood stained in this way, it is assumed that cells and tissues of similar composition react in the same way, as staining is a chemical reaction. Red cells, nucleated red cells including both cell and nucleus, and fibrin stain blue; white cells and blood platelets do not take the stain. From this it may be concluded that red cells and white cells are of different origin, that platelets do not have their origin in the nucleus of the red cells, and that fibrin has the same composition as the red cells.

These conclusions may throw some light on the processes of coagulation of the blood and certain phases of hemophilia leading to pernicious anemia. According to this line of reasoning, it may be possible that the origin of agglutinins in typhoid is in the red blood cells.—M. W. C.

A Method of Demonstrating Bacteria in Urine by Means of the Centrifuge. With Some Observations on the Relative Value of Examinations by Culture or Stained Sediment. E. G. CRABTREE. (Surg., Gyn., and Obstet., 1916, **22**, 221–224.)

The method consists in slow centrifugation to remove the heavier sediment, then rapid centrifugation until the urine is clear in order to throw the bacteria out of suspension. C. calls attention to the danger of mistaking smegma for tubercle bacilli, guinea pig inoculation being the final test for infection with tubercle bacilli. The author thinks inconsistent results are obtained because of lack of uniformity in culture media, etc., while organisms such as *B. coli* may overgrow the others. Microscopical examination assists in determining the degree of infection, and predominating organisms, and if there is a mixed infection helps to determine cultural method to be used.

Comment. The author does not mention the use of Petroff's method for direct culturing of tubercle bacilli or the necessity of using the antiformin method where T. B. is suspected and other infection already exists.—C. P. B.

A Rapid Method of Counting Living Bacteria in Milk and Other Richly Seeded Materials. W. D. FROST. (Journ. A. M. A., 1916, **66**, 889–890.)

A detailed account of the procedure is given. The method is essentially as follows:

"One-twentieth cubic centimeter of milk is mixed with standard nutrient agar and spread over a definite area of a sterile glass slide. When the agar is hard, this little plate culture is put in the incubator for about six hours under conditions which prevent evaporation. It is then dried, given a preliminary treatment to prevent the agar from firmly binding

the stain, stained, decolorized and cleared. When this dried and stained plate culture is viewed under the microscope, the little colonies are definitely stained and appear highly colored on a colorless or slightly colored background. These colonies can be readily counted and the number of bacteria per cubic centimeter calculated."—G. H. S.

Counting Bacteria by Means of the Microscope. R. S. BREED and J. D. BREW. (N. Y. State Sta. Tech. Bul. 49, pp. 31, pls. 2, figs. 5.)

This bulletin contains the results of tests of this method which have been made since those published in an earlier bulletin of the station (N. Y. Dept. Agr. Expt. Sta. Bul. 373, pp. 1–38 (1914)). A general description is given of the technique employed in applying this method to milk, the various processes involved being discussed with reference to possible errors. The result of this investigation are indicated by the following quotations from the authors' summary:

"The results obtained from the examination of samples of milk collected in clean test tubes containing preservatives indicate that just as accurate counts of the number of bacteria present can be made from such samples as can be made if the samples are collected in sterile tubes and iced. . . .

"Capillary pipettes have been found to be more satisfactory for the measurement of 0.1 cc. quantities of milk than standardized wire loops.

"Faulty calibration of pipettes has been found to be a serious cause of error. Allowance must be made for the adhesion of a certain quantity of milk to the pipette if accuracy of measurement is to be secured.

"It has been found that sterilization of pipettes is an unnecessary refinement of technique and that a single pipette may be used for making preparations from a long series of samples, provided it is carefully cleaned in glass-cleaning solutions after each day's use and also cleaned by rinsing in fresh, clean tap water after using in each sample and before passing to the next sample. Carelessless in cleaning pipettes causes marked errors in counts.

"Growth of bacteria has been found to take place in the drops of milk as they dry so that it is important that these be prepared either from samples containing preservatives or that the milk be dried quickly. No growth was detected in the dried films even after incubation in a moist, 37°C. incubator for one to four days.

"The claim made by some that bacteria are removed when the fat drops are dissolved by solvents does not seem to have any foundation in fact. The dried milk-solids-not-fat appear to act as a practically perfect fixative, no detectable mechanical loss of bacteria taking place when the fat 'rops are removed. On the other hand, serious errors in count are introduced where the bacteria are stained in the milk before the dried films are prepared, because in this way the bacteria are not always sufficiently stained to make it possible to detect the full number present. Where the fat drops are left in the films, even though these be spread out so as to be in a very thin layer, they tend to obscure bacteria and so lower the count. . . .

"Microscopical methods of examining dried milk-films are of value for two purposes: (a) They may be used for the rapid examination of milk in order to grade it according to its bacterial quality, both the number and the character of the bacteria present being taken into account. A microscopical examination permits a fairly accurate guess as to the probable plate count which will be secured from a given sample of milk. (b) They are also useful as research methods, the microscopical method being the only known method which permits a count of the number of individual bacteria. Microscopical counts of the number of isolated individual bacteria and compact clumps present in milk give figures which compare well with those obtained where petri plate methods of counting are used."—H. L. L.

MEDICAL BACTERIOLOGY

Foot and Mouth Disease in Man. R. L. SUTTON and A. O'DONNELL. (Journ. A. M. A., 1916, **66**, 947–949.)
Report of a case.—G. H. S.

Early Tuberculosis of the Cervix. T. S. CULLEN. (Surg., Gyn., and Obstet. **22**, 261.)
Tuberculosis of endometrium and cervix. Patient 25 years of age. Condition rare.—C. P. B.

Some Fatal Ear Cases in the Writer's Practice. O. D. STICKNEY, M.D. (Jour. of Ophth., Otol., and Laryngol., **21**, 189–204.)
Eight cases reported. Five had meningitis following otitis. Pneumococci were isolated from spinal fluid in one case, streptococci from another.—C. P. B.

The Choroidal Tubercle in Tuberculous Meningitis. J. F. BREDECK, M.D. (Am. Jr. Ophthalmol., **23**, 1–8.)
Choroidal tubercles may be found in 2 per cent of the cases of tuberculous meningitis if careful, daily search is made.—C. P. B.

The So-Called Primary Tuberculosis of the Conjunctivita and the Conjunctival Tuberculosis of Lupus Patients. K. K. K. LUNDSGAARD. (Am. Jr. of Ophthal., **33**, 54–59.)
Of 48 patients 19 had primary conjunctival tuberculosis; 29 lupus patients had conjunctival tuberculosis. Former believed to be endogenous and the latter ectogenous.—C. P. B.

An Unique Lesion of the Heart in Systemic Blastomycosis. T. B. HURLEY. (Jour. Med. Res., 1916, **33**, 499–502.)
A report of an autopsy of a case of systemic blastomycosis in which the musculature of the heart was extensively involved. This is, according to the author, the second case of its kind to be reported. No cultural studies are reported.—H. W. L.

Practical Points in the Prevention of Asiatic Cholera. ALLEN J. McLAUGHLIN. (Bost. Med. and Surg. Jour., 1916, **174**, 483.)

The author describes a rapid method of testing for chronic carriers of the cholera vibrio among immigrants. His steps are: inoculating peptone solution, streaking out on agar, and agglutination, with the possible use of Goldberger's enrichment solution. One hundred to 150 stools a day can be tested by one worker.—E. C. L. M.

Diphtheria in Manila. A. P. GOFF. (Journ. A. M. A., 1916, **66**, 941.)

As a result of a small outbreak of virulent diphtheria the Bureau of Science took more than 7000 throat cultures, finding 600 (or 9 per cent) positive for diphtheria.

Of the carriers found, 4 per cent developed symptoms of diphtheria.
G. H. S.

The Etiology and Treatment of Rat-Bite Fever. W. TILESTON. (Journ. A. M. A., 1916, **66**, 995–998.)

A case of rat-bite fever is reported. Organisms were found in the blood by darkfield examination which closely resembled *Streptothrix muris-ratti.* These organisms were to be found only during the febrile paroxysm, examinations made during the intervals being uniformly negative.

The administration of salvarsan was followed by a cessation of the paroxysms.—G. H. S.

Pathogeny of Diabetes and Fecal Disinfection. G. D. PALACIOS. (Medical Record, 1916, **89**, 543–551.)

The pathogeny of diabetes mellitus is a fecal putrefaction and a fecal reabsorption of ammoniacal and acid character. Although fasting and a very restricted diet are the best dietetic treatment of diabetes, fecal disinfection is both preventive and curative.

Intestinal putrefaction may be overcome in some cases by the acidogenous *Bacillus bulgaricus.* In the tropical Atlantic region, an absolute intestinal disinfection is effected by the ingestion of *Micrococcus oxycyanogenes.*—M. W. C.

The Etiology of Scarlet Fever. F. B. MALLORY, and E. M. MEDLAR, (Jour. Med. Res., 1916, **34**, 127–130.)

In a short communication, the authors describe finding, in the crypts of the tonsils, and in erosions of the epithelium of the tonsils, fauces, soft palate, uvula, trachea and lung of a child dying of scarlet fever on the second day following the eruption, clumps of Gram-positive bacilli, together with streptococci. Similar organisms were found in four other cases. The organism is best grown anaerobically on 3 per cent glycerin, 0.5 per cent glucose serum-agar. The authors believe that the organism dies out rapidly or is overgrown by streptococci which accounts for previous failures. In view of the lack of animal experiments

and the small number of cases examined, the work does not appear to be conclusive.—H. W. L.

The Etiology of Rocky Mountain Spotted Fever. S. B. WOLBACH, (Jour. Med. Res., 1916, **34**, 121–127.)

Guinea-pigs inoculated by ticks infected with the virus of spotted fever, show definite pathological changes characteristic of this disease. The author finds in the diseased tissue an organism, agreeing in most respects with that of Ricketts, which he feels justified in calling a bacillus. This organism is described as Gram-negative, resembling somewhat *B. influenzae* but stained bluish by Giemsa, in contrast to most bacteria. All attempts to cultivate the organism have failed.

H. W. L.

Studies on Treponema pallidum and Syphilis. II. Spirochaeticidal Antibodies against Treponema pallidum. H. ZINSSER, and J. G. HOPKINS. (Jour. Exp. Med., 1916, **23**, 323–328.)

Cultures of the *Treponema* were grown on a new medium consisting of inspissated egg in tubes filled with broth serum mixtures. This method makes it possible to obtain clean antigen, unmixed with tissues detritus, a disadvantage incident to tissue cultures. The authors believe that their experiments have shown that the serum of rabbits and sheep immunized with cultures of *Treponema pallidum* acquire spirochaeticidal properties for these culture spirochaetes. The normal serum of these animals also possesses spirochaeticidal action if used in sufficient quantities, and the action of the immune serum represents probably an increase of normal antibodies. Both normal and immune spirochaeticidal properties are destroyed by heating to 56°C. but the serum can be reactivated by the addition of fresh normal serum of the same species, insufficient in amount to exert a spirochaeticidal effect by itself. The structure of these spirochaeticidal bodies appears to be analogous to that of the well-known bactericidal antibodies known to exist in antibacterial sera. It is pointed out by the authors that these results apply to culture spirochaetes.—G. B. W.

III. The Individual Fluctuations in Virulence and Comparative Virulence of Treponema pallidum Strains Passed Through Rabbits. HANS ZINSSER, J. G. HOPKINS, and M. McBURNEY. (Jour. Exp. Med., 1916, **23**, 329–340.)

Rabbits were inoculated with strains from human cases with the purpose of studying differences in racial and acquired virulence. The authors found no difference in pathogenicity between the different strains, although they were isolated from various lesions, and, further, these strains show no consistent change in rabbit pathogenicity during progressive rabbit passage (21 generations in one case). Variations in the lesions produced, and also in the incubation time are probably due to variations in technique.—G. B. W.

IV. The Difference in Behavior in Immune Serum between Cultivated Non-Virulent Treponema pallidum and Virulent Treponemata from Lesions. HANS ZINSSER, J. G. HOPKINS, and M. McBURNEY. (Jour. Exp. Med., 1916, **23**, 341–352.)

Although antibodies can be produced by the immunization of animals with cultivated *Treponema pallidum*, and although these antibodies exert specific agglutinative and treponemicidal action upon the culture organisms, they possess, at least in the concentration so far obtained by the authors in rabbits and sheep, practically no action on virulent treponemata obtained directly from lesions.—G. B. W.

An Experimental Study of Parotitis (Mumps). MARTHA WOLLSTEIN. (Jour. Exp. Med., 1916, **23**, 353–375.)

Cats injected in the parotid gland and testicle with a bacterial sterile filtrate of the salivary secretion of children in the active stage of parotitis, or mumps, can be made to develop a pathological condition having several points of resemblance to the condition present in mumps in human beings. Definite changes in the temperature, blood leucocytes, and inoculated organs take place after an incubation stage of from 5 to 8 days. These pathological changes are intensified by successive transfers through a small series of cats of the extract and emulsion of the parotid gland and testicle previously inoculated. These changes can also be prevented or reduced when the extract or emulsion is previously incubated with blood serum obtained from a cat which has survived inoculation. Normal serum, on the other hand has no such inhibiting effect. Whether the filtered salivary secretion contains a microörganism and, if so, whether it is the specific microbic cause of parotitis, or mumps, remains to be ascertained.

G. B. W.

The Etiology, Mode of Infection, and Specific Therapy of Weil's Disease (Spirochaetosis icterohaemorrhagica). R. INDA, Y. IDO, R. HOKI, R. KANEKO and H. ITO. (Jour. Exp. Med., 1916, **23**, 377–402.)

In the course of their investigations of that endemic disease of portions of Japan, which agrees clinically with Weil's disease, so called, the authors discovered a spirochaetal microörganism which they name *Spirochaeta icterohaemorrhagiae*, and which they believe to be the cause of the disease. These spirochaetes live in the blood outside the cellular elements and in various organs and tissues. Infection is supposed to be by way of the alimentary canal or it may enter through the skin. The spirochaetes are excreted through the urine. The serum of convalescents possesses bactericidal and bacteriolytic properties and recovery from the disease confers a lasting immunity. Treatment with salvarsan appears to offer promising possibilities, while passive immunization with immune serum has already given gratifying results. Many excellent plates are appended.—G. B. W.

Bacteria Associated with Certain Types of Abnormal Lymph Glands.
J. C. TORREY. (Jour. Med. Res., 1916, **34**, 65–81.)

With a view to substantiating the claims of various authors that a diphtheroid bacillus is the causative agent in Hodgkin's disease, the author cultured 40 abnormal lymph glands, including 10 cases of Hodgkins. Three distinct groups of aerobic diphtheroid bacilli, one anaerobic group, and various other types of organisms were found and such a diversity of pathological conditions as to preclude the possibility of attaching importance to any one type as the cause of Hodgkin's disease. Serological reactions and animal inoculations failed to show any specificity.

The finding of the anaerobic diphtheroid type in 100 per cent of the cases of Hodgkin's as well as in various other conditions, although interesting, the author does not believe is of any importance as throwing light on the cause of the disease in question, and only emphasizes the need of caution in accepting uncontrolled results as conclusive evidence.—H. W. L.

The Diagnosis of Genitourinary Tuberculosis. J. W. CHURCHMAN. (Medical Record, 1916, **89**, 511–513.)

The mode of entrance of tubercle bacilli into the urine is not definitely proved, but it seems probable that the normal kidney is permeable for the tubercle bacillus as well as for other organisms. That infection does not extend upward from bladder to kidney is a well-established fact.

The most reliable sign in diagnosing renal tuberculosis is the presence of tubercle bacilli in the urine, though in rare cases there may be tuberculosis of the kidney with no demonstrable tubercle bacilli in the urine. In such cases the granules described by Much may be worthy of attention. These granules are interpreted by him to represent types of the tubercle bacillus which do not possess the ordinary acid-fast character. Although it is impossible to say what the significance of the Much granules is, it is true that they were present in the urine in a case of tuberculous kidney where tubercle bacilli were not found in the urine.—M. W. C.

Relapsing Fever in Serbia. J. RUDIS-JICINSKY. (New York Med. Jour., 1916, **103**, 643–645.)

Hundreds of cases of relapsing fever occurred in Serbia during the past winter.

In many cases examination of the blood revealed *Spirillum obermeieri*. This organism was filamentous, of spiral form, much elongated, and in motion followed its long axis. It was about four times the diameter of a red blood corpuscle. The organism was aerobic. It could be stained easily with anilin colors in dry blood, but was not found in other fluids or secretions of the body.

The stage of the disease at which the spirochaete could be found in the blood was not always the same.

Inoculation of blood containing spirochaetes into rabbits conveyed the disease to these animals. After death spirochaetes were present in all the organs, but could not be cultivated upon artificial media.

In nearly every case, the louse could be considered as the carrier of the infection, and the prevention of lousiness was a necessary step in eradicating the disease.—M. W. C.

Colon Bacillus Infection of the Bladder. R. T. MORRIS. (New York Med. Jour., 1916, **103**, 631–632.)

Many cases of cystitis of obscure origin may be due to the colon bacillus, as this bacillus is sometimes found upon examination of the urine, and conditions similar to cystitis have been induced in experimental animals by injecting *Bacillus coli* into the bladder.

The differences in the type of infection are probably due to the particular strain of colon bacillus causing the condition, for there is a wide variation among the members of this group. According to the recent work of Rosenow it may even be possible that unusual types of cystitis are caused by other bacilli, which have assumed the form of *B. coli*. Uncertain action of vaccines in cases of colon bacillus infection is perhaps due to such a variation in the infecting organism.

Colon bacilli may be responsible for any of the widely different manifestations of cystitis.

The mode of entrance of the colon bacillus into the bladder probably differs with varying conditions.—M. W. C.

Studies on Diphtheria. II. The Treatment of Diphtheria Carriers by Tonsillectomy. H. O. RUH, M. J. MILER and R. G. PERKINS. (Journ. A. M. A., 1916, **66**, 941–943.)

The termination of the carrier condition through the use of biological or chemical methods did not meet with great success.

In a series of 19 cases tonsillectomy was performed. The average duration of the carrier state before operative treatment was resorted to was 31 days. The average duration of release from quarantine after the tonsillectomy was 8 days.

In all cases cultures made from the crypts after excision yielded *Bacillus diphtheriae* in nearly pure culture although surface cultures were frequently negative.—G. H. S.

Bacteriological Work at the American Ambulance. ORVILLE F. ROGERS and GEORGE BENET. (Bost. Med. and Surg. Jour., 1916, **174**, 418.)

The authors report on the bacteriological work done in the Harvard University Service of the American Ambulance from April 1 to July 1, 1915.

From 100 men examined 28 showed gas-producing organisms. Of these 28, 18 were obtained in pure culture and run through sugar media. The authors conclude that sugar media are not suitable for the differentiation of the gas-producing bacilli.

"The majority of cultures showed staphylococci either alone (30)

or with other organisms (58). Forty cultures showed an anaerobic growth of other than gas producers. Other organisms seen were: pneumococcus (11), streptococcus (9), pyocyaneus (5), and varieties of other than gas producers (25)."—E. C. L. M.

The Use of Kaolin to Remove Diphtheria Bacilli from the Nose and Throat. B. RAPPAPORT. (Journ. A. M. A., 1916, 66, 943–945.)

A study of the use of kaolin in 100 cases, 96 being diphtheria patients and 4 carriers.

Kaolin, thoroughly dried and finely powered, is distributed over the surfaces to be treated. In young children application can best be made to the nasal mucous membrane even though the bacilli are in the pharynx. Some of the kaolin will work its way into the throat but the greater part will remain in the nose. Before a second treatment the kaolin already applied and now holding organisms should be removed by a mild alkaline spray.

Six treatments per day at two hour intervals are given. With older patients the kaolin is swallowed, four half teaspoonful doses at two hour intervals six times during the day.

The action of kaolin appears to be wholly mechanical, no bactericidal action being evident.

The nose may be freed of bacilli much more readily than the throat.

Compared with 100 consecutive cases dismissed before the use of kaolin, the treatment effected a percentage reduction of hospital stay of 23.4.

Various pathological conditions, as adenoids and diseased tonsils, interfere with the action of kaolin. In such cases surgical treatment is required.—G. H. S.

The Practical Value of Guinea Pig Tests for the Virulence of Diphtheria Bacilli. JOHH A. KOLMER, SAMUEL S. WOODY. EMILY L. MOSHAGE. (American Jour. Diseases of Children, 1916, 4, 257–268.)

The paper is based upon the results obtained with the guinea pig test for virulence on 1054 diphtheria cultures. The method employed consists of isolation of the bacilli upon slants of Loeffler's blood serum media, subculturing in 0.2 per cent glucose broth with a reaction of plus 0.8, incubation at 37°C., for seventy-two hours and injection subcutaneously in the median line of a pig weighing from 250 to 300 grams with a dose corresponding to 0.5 per cent of the weight of the animal expressed in cubic centimeters. The total amount injected is brought up to 4 cc. The animal is observed for four days and the development of a typical local inflammation with toxemia is regarded as diagnostic If in doubt a second pig is inoculated and at the same time is given 500 units of diphtheria antitoxin. 4 cc. of a good 24 hour culture grown upon a tube of Loeffler's blood serum washed off in 10 cc. of salt solution can also be used for injection and has the advantage of saving 48 hours in time. Granular and barred types were found virulent in about 70 per cent of cultures from throat, nose and ear, long

solid forms in about 42 per cent of cultures, while short solid types were uniformly found to possess no virulence. The authors, especially emphasize the importance of tests for virulence in recovery cases before dismissal and in suspected carrier cases.—R. M. T.

A Preliminary Report on Pneumonia in Children, with Special Reference to its Epidemiology. GODFREY R. PISEK and MARSHALL C. PEASE. Am. Jour. Med. Sc., 1916, **151**, 14.

In an analysis of 1000 cases of pneumonia, not including those cases secondary to other infectious diseases, the authors found a mortality of 34.5 per cent in children under six years of age in the Babies' Wards of the New York Post-Graduate Hospital. The series contained 445 cases classed as bronchopneumonia which occurred chiefly during the first two years of life, and were relatively uncommon after the third year. The lobar form also occurred more frequently during the first two years, and was the type usually found after the third year if the terminal and secondary infections following other diseases are excluded. The highest mortality was found in the first year of life with both forms, but relatively less frequently with the lobar. No evidence was found of either epidemic or house infection in studying the cases. The authors felt that the broncho- and lobar types formed rather distinctive groups clinically, their conclusions being based upon both pathological and bacteriological differences. For bacteriological study the materials were taken from the upper part of the larynx by means of a bent applicator. In 23 cases of lobar pneumonia, with sputum virulent for mice, all showed Gram-positive diplococci predominating in nearly every case with a few streptococci and staphylococci. In 10 cases of mild bronchopneumonia with sputum which seemed virulent to mice, the predominating organism in 5 cases was the streptococcus. 2 cases each showed staphylococci and the influenza bacillus, and in 1 case, tubercle bacilli with other organisms. In 4 cases a few pneumococci were present. In 8 cases of bronchopneumonia with sputum virulent to mice, smears showed large numbers of pneumococci, in addition to large numbers of other organisms, chiefly streptococci and staphylococci. Bacteriologically, the authors consider bronchopneumonia as being a mixed infection, or an infection chiefly with one type of organism other than the pneumococcus. This differentiates this form from the lobar type, which is due chiefly or entirely to the pneumococcus. A study was made of the types of pneumococci occurring in a group of 48 clinical cases of pneumonia, which gave conclusive results. Of these, 28 cases were classified as lobar and 20 cases as bronchopneumonia. For this purpose the Dochez-Gillespie grouping of pneumococci was followed, using specific sera for Groups I and II prepared by the Rockefeller Institute, and the methods recommended by them. The series gave the following results: Group I, 11 cases; Group II, 14 cases; Group III, 4 cases; and Group IV, 19 cases. Cases clinically classed as lobar pheumonia showed pneumococci Groups I and II, decidedly predominating; while more than

half of the clinical bronchopneumonias fell in Group IV. The mortality rate according to groups was as follows: Group I, 9 per cent.; Group II, 36 per cent.; Group III, 25 per cent.; and Group IV, 21 per cent. Eleven strains of pneumococci taken from the throats of children showing no lung involvement fell in Group IV.—L. W. F.

PALEONTOLOGY

Mesozoic Pathology and Bacteriology. Roy L. Moodie. (Science, 1916, **43**, 425–426.)

Attention is called to this rather unusual, though fascinating and important branch of bacteriology. The author feels that we have convincing proof of the existence of fungi and bacteria in coprolites, and of pathologic conditions in various fossil tumors and fractures. —C. M. H.

PLANT PATHOLOGY

Further Studies in the Rôle of Insects in the Dissemination of Fire Blight Bacteria. V. B. Stewart and M. D. Leonard. (Phytop. 1916, 152–158.)

From observations made throughout several seasons the writers believe that all the sucking bugs of the nursery bear infection. Experiments were conducted by caging upon trees insects which had on their bodies organisms from a pure culture of *Bacillus amylovorus*. The various species of flies are thought not to be active agents in transmitting infection though they may be important in carrying the organism to blossoms or to wounds. The experiments included the following suspected carriers: *Pollenia rudis, Empoasca mali, Psylla pyricola, Plagiognathus politus, Sapromyza bispina.*—F. L. S.

Citrus Canker. F. A. Wolf. (J. Agr. Res. 6, 69–99.)

A serious citrus disease has recently been introduced into the Gulf States, known as citrus canker. The primary cause is *Ps. citri* Hasse, an organism with a single flagellum, shown by the writer to have the group number Ps. 221. 3332513. It attacks both twigs and leaves. Fungi of the genera *Phoma, Fusarium* and *Gleosporium* have been found associated with this organism, although the *Phoma* is the only one found to be notably active in disintegration of the tissues. The only method of control recommended is by means of quarantine and thorough destruction of diseased trees.—H. J. C.

BIOLOGICAL VARIATIONS OF BACTERIA[1]

I. INDUCED VARIATIONS IN THE CULTURAL CHARACTERS OF B. COLI

M. R. SMIRNOW

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Variations in the biological characteristics of the various members of the Colon-typhoid group have been reported by numerous investigators. These range from mere observations as to peculiarities in the fermentative or other biological tests up to actual mutations as interpreted by De Vries.

The most important observations along the latter line are reported by Massini (1907), who isolated a type of *B. coli* he called *"B. coli-mutabilis."* In his observations, he found this organism would produce flat colorless colonies on Endo's medium, if transplanted every twenty-four hours. When transplanted at a later period, however, it would produce nodular shaped colonies which became red. The colorless colonies always gave rise to colorless colonies when transplanted not later than twenty-four hours, whereas the red colonies once obtained, never gave rise to any but red colonies irrespective of the time of transfer. The knob like colonies appeared only on lactose media though other carbohydrate media were used. He observed a single reversion from the red to the colorless type, which, however, could not be repeated.

Burk (1908) reports the isolation of a similar mutant and records careful observations which were continued over a period of five months.

Of the more interesting reports in literature on modifications of *B. coli* and allied organisms may be mentioned those of Peck-

[1] Read in part, before the meeting of the Society of American Bacteriologists, held at Philadelphia, December, 1914.

ham, Herter, Penfold, Twort, Manfredi and others. The work of the first of these investigators will be mentioned below, in conjunction with the experiments of the writer.

Herter (1910) has shown that sodium benzoate in weak glucose broth considerably inhibits the fermentative activities of *B. coli*, whereas other biological features are but slightly affected. Such action is entirely prevented by the addition of calcium carbonate. He has also shown that there are no gas producers in food stuffs preserved with sodium benzoate, though 22 of 28 samples contained bacteria of some sort. Penfold (1911) has shown similar action in the case of sodium acetate on *B. coli*, *B. enteritidis* and *B. paratyphi* with diminishing and total disappearance of gas formation in the sugars, though the organisms were still capable of producing gas from the corresponding alcohols. This indicated an inhibition or destruction of the enzyme, invertase, without effect upon the gas producing power. Twort (1907) has shown that *B. typhi*, *B. paratyphi* and *B. dysenteriae* when continuously grown in saccharose media will ultimately ferment saccharose. Manfredi (1889) states that fat-containing media impair the vegetative energy of bacteria.

The observations here reported were undertaken in connection with a series of experiments on the biological variations of bacteria, which the writer intends publishing in sections whenever a sufficient amount of interesting material is accumulated to warrant it. Twenty-one different strains of the various bacilli of the colon-typhoid group were used, in the study but this report is confined only to the *B. coli*, of which seven different strains were experimented on. All of these strains were obtained from the American Museum of Natural History, New York, through the kindness of Prof. C.-E. A. Winslow, and were the stock nos. 19, 44, 45, 46, 52, 57, and 95. The bacteria were subjected to continuous growth at 37.5°C. in 3 per cent glucose, 4 per cent sodium chloride and 1.5 per cent sodium sulphate broth. They were also grown in plain broth and then exposed to the action of phenol in the following manner. The culture was first inoculated into 9 cc. of plain nutrient broth

and incubated for three or four days, at which time 1 cc. of 7.5 per cent of phenol was added to the culture. The phenol exposure was limited to two to three minutes at the beginning of the experiment and then the time was gradually ncreased with each transfer until thirty or more minutes time was attained. The exposed culture was then reinoculated into plain broth by pouring over a small quantity (0.5 to 1 cc.) from it. Continuous growth of the *B. coli* in 0.75 per cent of phenol broth was also tried, beginning with 0.25 per cent, with the same general results. The transplanting in all media was carried out every three or four days over periods varying from one to three months, thus allowing from ten to thirty transfers. The experiments were repeated two or three times to assure constant and uniform findings.

Control cultures were carried on in plain broth throughout the experiment. It might be stated at once that there were very slight variations between the original stocks and these control cultures, no more than would be expected as normal variations. These were seen as slightly increased or decreased amounts of gas or acid formation, differences in time of coagulation, or slight changes in the growth on potato. At no time, however, were the biological characteristics markedly changed nor enzyme production completely inhibited simply by continual passage through broth.

The accompanying tables show the results obtained in some of the more typical series of experiments. These tabulations were all made at seventy-two or ninety-six hours after inoculation and were verified again, especially those on potato and in milk, after a week or ten days growth. The tests for indol were made as described below after seven days growth.

TABLE I

Marked effect of glucose on B. Coli No. 45. Phenol gave a moderate amount of variation particularly in milk and gas production, whereas both sodium chlorid and sulphate showed but slight effect

B. COLI NO. 45	POTATO		MILK		GLUCOSE		LACTOSE		MALTOSE		SACCHAROSE		DEXTRIN		MANNITE		FERMENTATION TUBE	
	Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Control.........	+++	+++	++	+++	++	+++	++	+	++	++	++	++++	++	++	++	++	+++	+
Glucose.........	−	−	−	−	−	−	−	−	−	−	−	−	++	++	−	−	−	−
Phenol.........	+++	++	++	++	++	+++	++	+	++	+++	++	++	+++	+++	+	++	+++	+
Sodium chlorid...	++	+++	++	+++	++++	++++	++++	++++	++++	++++	+++	++	++++	++++	++++	++++	+++	+
Sodium sulphate..	++	+++	++	+++	++++	++++	++++	++++	++++	++++	+++	++	++++	++++	++++	++++	+++	++

TABLE II

Marked effect of glucose, two different series. The power of producing acid in No. 2 is apparently not effected. Moderate action of phenol and the sodium salts, especially in regard to gas formation

B. COLI NO. 44	POTATO		MILK		GLUCOSE		LACTOSE		MALTOSE		SACCHAROSE		DEXTRIN		MANNITE		FERMENTATION TUBE	
	Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Control.........	+++	+++	+++	+++	++	+++	++	++	++++	++++	+++	++++	++	++	++	+++	+++	+++
Glucose... (1)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Glucose... (2)	−	−	+	+	+++	−	+++	−	+++	−	++	+	++	−	++	++	++	+
Phenol... (1)	+++	+++	++	+++	++	−	++	+	++	++	+	−	+++	+	+	+	+	−
Phenol... (2)	+++	+++	++	−	++	−	++	−	++	+	+++	++	++	++	+++	++	+++	++
Sodium chlorid...	+++	+++	+++	+++	−	−	++	++	++	++	+++	++	++	++	+++	+	+++	+
Sodium sulphate..	+++	+++	+++	+++	−	+	++	++	++	++	+++	++	++	+	++	+	+++	+

TABLE III

Marked effect of glucose and phenol, slight effect of the sodium salts

B. COLI NO. 45	POTATO		MILK		GLUCOSE		LACTOSE		MALTOSE		SACCHAROSE		DEXTRIN		MANNITE		FERMENTATION TUBE	
	Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Control	+++	++++	++++	++++	++	++++	++	+++	++++	+++	++	++	+++	+++	+++	+++	++++	+++
Glucose (1)	++	−	++	+++	−	−	−	−	++	++	−	−	++	++	++	++	++	+++
Glucose (2)	+	+	++++	++++	++	−	++	−	++	++	++	−	++	−	++	−	−	−
Phenol	++	−	++	++	+	−	++	++	++	+++	++++	+	+++	+++	+++	+	++++	+++
Sodium chlorid	+	++	++	++	++++	++++	++++	++++	++++	++++	++++	+	++++	+++	++++	+++	++++	+++
Sodium sulphate	+	+	++	+++	++++	++++	++++	+++	++++	+++	++++	++	+++	+++	++++	+++	++++	+++

TABLE IV

No effect of glucose, slight effect of phenol, moderate effect of sodium salts, particularly upon the fermentative qualities

B. COLI NO. 46	POTATO		MILK		GLUCOSE		LACTOSE		MALTOSE		SACCHAROSE		DEXTRIN		MANNITE		FERMENTATION TUBE	
	Br. Gl.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Control	+++	++++	+++	++++	+++	+++	++++	++++	++++	+++	++++	+++	++++	++++	++++	+++	++++	+++
Glucose	+	−	+++	++++	+++	+++	++++	+++	++++	+++	++++	+++	++++	++++	++++	+++	+++	+++
Phenol (1)	−	−	+++	+++	++	++	+++	−	++	−	+	−	++	−	++	−	+	−
Phenol (2)	+	++	++	++	++	+	+++	−	+	−	+	+	++	−	+	−	+++	++
Sodium chlorid	+	++	++	++	+++	+++	++++	++++	+	−	++	−	++	−	+	−	+++	−
Sodium sulphate	++	++	++	+++	++++	++++	++++	++++	+	−	++++	+	+	−	+	−	+++	+++

TABLE V

Marked effect of phenol, and slight effect of glucose, none of sodium salts

B. COLI NO. 10	POTATO		MILK		GLUCOSE		LACTOSE		MALTOSE		SACCHAROSE		DEXTRIN		MANNITE		FERMENTATION TUBE	
	Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Control	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	++	+++	++	+++	+++	+++	+++	+++
Glucose	-	-	++	+	++	++	++	+	++	++++	++	+	++	+	++	++	+	-
Phenol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sodium chlorid	+++	+++	+++	+	+++	+++	+++	+	+++	++	+++	+++	+++	+++	+++	++	+++	++
Sodium sulphate	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

TABLE VI

Slight effect of sodium salts, none of either glucose or phenol

B. COLI NO. 52	POTATO		MILK		GLUCOSE		LACTOSE		MALTOSE		SACCHAROSE		DEXTRIN		MANNITE		FERMENTATION TUBE	
	Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Control	+++	+++	++	+++	++	+++	++	+++	+++	+++	++	+	++	+++	++	+++	+++	++
Glucose	++	-	++	+++	++	++	+++	++	+++	+++	++	++	+++	+++	+++	+++	+++	++
Phenol	+++	-	+++	+	+++	+	++	++	+++	+++	++	++	++	++	-	-	++	++
Sodium chlorid	-	+	++	++	+++	+++	+++	+++	+++	+	+++	+++	++	++	++	-	++	+
Sodium sulphate	-	+	++	++	+++	+++	+++	+++	-	-	+++	+++	++	++	++	+	++	++

TABLE VII

Slight effect of all agents, principally the sodium salts

B. COLI NO. 57	POTATO		MILK		GLUCOSE		LACTOSE		MALTOSE		SACCHAROSE		DEXTRIN		MANNITE		FERMENTATION TUBE	
	Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Control	+++	++	++	+++	++	+++	++	+++	+++	+++	++	+	++	+++	++	+++	+++	++
Glucose	+++	++	++	+++	+	++	+++	++	++	+++	++	++	+++	+++	+++	+++	+++	++
Phenol	++	+++	+	+	+	+	++	+	-	-	++	++	++	++	+	+	++	++
Sodium chlorid	+	+	++	++	+++	+++	+++	+++	-	-	+++	+++	+++	++	++	-	++	+
Sodium sulphate	+	+	++	++	+++	+++	+++	+++	-	-	+++	+++	++	++	++	+	++	++

CHANGES IN MORPHOLOGY

During the course of experimentation several of the strains of B. coli showed a change in morphology, the individual organisms becoming two and three times the length of those in the controls, somewhat wider and more vacuolated. This however was not constant, as often on the very next sub-culture they would assume their usual morphological appearances. The only other thing noted under this head was the decrease in motility, which was more marked in the phenol broth than in the other media. Very little importance should be attached to this, however, since some of the strains were hardly motile to begin with, and again, too few observations were made to permit of absolute statements.

GROWTH ON POTATO

Glucose seemed to have a special effect upon the character of growth of B. coli on this medium. Five of the seven treated strains, showed at best only a very light yellow color or a slight brownish growth on ordinary potato with practically no discoloration of the medium. Very frequently indeed, the glucose affected organisms would give the typical "invisible" growth seen with the B. typhi. Both the original stock and the broth control showed the characteristic colon growth on this medium. This change was noted so many times that an explanation based on differences in the composition of the potato can be excluded. Three of these five strains also showed this change after exposure to phenol. One strain of the B. coli, which did not change in this respect with either glucose or phenol, showed this same variation after growing in either sodium chloride or sodium sulphate broth.

ACTION IN MILK

Both phenol and glucose diminished the acid production and inhibited the formation of lab enzyme in three of the seven strains of the B. coli, either entirely or for a period of at least two weeks. These results were not seen with the use of the strong saline or sodium sulphate broth.

FERMENTATION OF SUGARS

The results obtained with these substances on *B. coli* with reference to variations in sugar fermentations can be best seen in the accompanying tables. The most striking changes here also were seen in those organisms exposed to glucose and phenol. The former completely inhibited both acid and gas formation in all the sugars tested in three different strains. In two others, glucose varied the amount of acid and gas formation, with an occasional complete inhibition in some of the sugars. Phenol inhibited these fermentations in all of the sugars in only one strain, and in four others, diminished this reaction to the point of inhibition at times only and in different carbohydrates. Sodium chloride and sodium sulphate had less effect than did phenol, giving usually slight variations in amount of acid or gas produced with an occasional inhibition.

Inhibition of all the sugar fermentations in any one experiment was almost always accompanied by inhibition of the usual changes in milk, the characteristic growth on potato, and the formation of indol. In other words, the most typical variations were those in which all acid or ferment production was inhibited.

VARIATIONS IN INDOL PRODUCTION

The production of indol is held by many bacteriologists to be as important a biological characteristic of *B. coli* as its fermentations of the sugars, and is even thought to be of greater importance in its differentiation. This reaction, however, even under normal conditions varies considerably in its quantity and time of appearance with most strains, and at times requires more delicate tests than the usual Salkowski method for its determination.

In the experiments here reported it appears that of the variations induced in *B. coli* that of indol production is the first to take place, often disappearing in the third or fourth culture in glucose broth. This does not hold however when the bacteria are grown in the other media, as evidenced below.

Each strain of *B. coli* was grown in plain broth as control, and in glucose, phenol, sodium chloride and sodium sulphate broth and on potato. Thirty-five sub-cultures were made in all. Indol was tested for after the 10th, 15th, 25th, and 35th transfers. The tests for indol were made by inoculating one loop of culture from the respective medium to which each strain was subjected into standard peptone solution, growing for seven days at 37°C. and then testing by the Salkowski method. All the tests were done at the same time using the same batch of peptone solution throughout the experiment.

The results were uniform for all strains and may be readily interpreted from the following table:

TABLE VIII

NUMBER OF TRANSFER	1	10	15	25	35
Control.................	+++	+++	+++	+++	+++
Glucose broth............	+++	−	−	−	−
Phenol broth.............	+++	++++	++++	++++	++++
NaCl broth*............	+++	++	++	+++	++
Na₂SO₄ broth*.........	+++	++	++	+++	++
Potato.................	+++	+++	+++	+++	+++

* Exposure to these substances gave variable results, at times an increase and at others a decrease in indol production.

All the controls, grown in plain broth, gave good indol tests even after the 35th sub-culture. Those grown in glucose broth gave none at the 10th sub-culture or thereafter. In phenol broth the property of indol production seemed to be somewhat increased, judging from the intensity of the reaction. Sodium chloride and sodium sulphate, and prolonged cultivation on potato practically exerted no influence, or if any, showed a slight inhibitory effect.

Experiments were then carried out to see how soon the property of indol production is interfered with by growth in 3 per cent glucose broth, and it was found that *B. coli* lost this property usually on the third and at times on the second transfer over a period of from seven to ten days. In one experiment sub-cultures were made every twenty-four hours with a total

disappearance of the indol tests in from forty-eight to seventy-two hours in all the strains.

The tests in these latter experiments were made in the culture tubes themselves, not transferring to the peptone solution, after seven days of growth. In order to exclude the possibility of interference with the indol test by the presence of the glucose, several cultures in both plain broth, and peptone, were made, and grown at 37°C. for seven days. Glucose was added to each of the cultures and they were then tested for indol. Positive tests were obtained in all cases, excluding any possibility of such interference by the presence of the carbohydrate. An interesting observation may also be mentioned at this juncture. Cultures of the organisms in plain broth of seven days growth to which phenol or sodium chloride were added showed a decided increase in the indol reaction in case of the phenol and a diminished reaction in the tubes to which the sodium chloride was added. In the interpretation of these tests comparison was made with controls. It may be possible that the presence of these substances intensifies or diminishes the color produced, the differences not being due to actual variations in the amount of indol formed. The different culture media themselves were tested for indol, after incubating for seven days, for the purpose of control and they were found negative.

Experiments were then carried out to determine the permanency of this change. The cultures in glucose broth after the 35th transfer were grown in plain broth, transplanting every day and tested on the seventh day of incubation. Four of the strains of *B. coli*; nos. 44, 45, 46 and 52 gave slight indol reactions on the third transfer, no. 46 gave a good positive on the fifth transfer, but the others took from five to ten more transfers before they could be called "+" or "++" positive. Nos. 57 and 95 took six transfers before a trace of indol appeared. No. 19, a very feeble indol producer in the control, remained negative up to the fifteenth transfer at which time the experiment was discontinued.

Investigations as to the agglutinability of these altered strains of *B. coli* were also made, but the work is too meagre and the

results too indefinite to be reported at the present time. The writer intends to continue work along this line, and also with respect to pathogenicity, which seems to suggest itself as a fruitful subject for investigation.

In summing up, it can be said that glucose and phenol, particularly the former, cause partial inhibition or total disappearance of acid and enzyme formation in some strains of *B. coli*. These changes together with the suspension of the production of indol and the characteristic colon growth on potato, makes the *B. coli* approach the *B. typhi* type. These changes have been noted time and again but in varying degrees, in those strains

TABLE IX

Changes produced by glucose and phenol in various strains of B. coli and the complete or incomplete reversion towards their previous biological characteristics. The completeness of the change and the incompleteness of the reversion in strain No. 95 is noteworthy, as such a change might be regarded in the light of a mutation instead of a variation

	POTATO		MILK		GLUCOSE		SACCHAROSE		FERMENTATION TUBE	
	Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas
B. Coli, No. 95										
Control......	+++	+++	+++	+++	++	+++	++	++	+++	+++
Glucose......	−	−	++	++	++	−	++	−	++	−
Reversion....	−	−	++	++	−	−	+	−	−	−
B. Coli. No. 19										
Control......	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Glucose......	−	−	−	−	−	−	−	−	−	−
Reversion....	−	−	+++	+++	+++	++	++	+++	+++	++
B. Coli. No. 44										
Control......	+++	+++	+++	+++	++	+++	+++	+++	+++	+++
Glucose......	−	−	−	−	−	−	−	−	−	−
Reversion....	+++	+	++	+++	+++	+++	++	Sl.*	+	Sl.
Phenol.......	+++	+++	++	+++	+	−	++	−	−	+
Reversion....	++	++	+++	+++	++	+	++	++	+	+
B. Coli. No. 45										
Control......	+++	+++	++	+++	++	+++	++	+++	+++	+
Glucose......	−	−	−	−	−	−	−	−	−	−
Reversion....	−	−	++	Sl.	+	+	+	+	+	+
Phenol.......	+++	++	−	−	++	+++	−	−	−	−
Reversion....	++	++	++	Sl.	+	+	+	+	+++	+

* Sl. = slight.

that are susceptible to variations, but for some unexplained reason cannot be regarded as altogether constant. Indol formation would invariably return when these altered bacteria were transplanted into plain broth at frequent intervals. Lab enzyme would also return in most of the altered strains but not invariably so. The same can be said of the fermentative properties. Very often, however, these characteristics appear to be entirely done away with, the change being permanent as far as could be made evident by sub-culturing into plain broth. (See Table IX.) In these cases observations were made up to two months after the last exposure to the influencing substance, making frequent transfers. There seemed to be no definite rule of reversion, and no relation between the reappearance of one enzyme and another. The reappearance of the fermenting enzymes in one sugar was not necessarily accompanied by those in other sugars. At times the fermentation of one sugar might have returned to nearly normal, while others might show little or no presence of gas with the same strain of *B. coli*.

ADDENDA

The work under this heading was undertaken as supplementary to the foregoing section for the purpose of verification and also in response to comments made upon the report of this paper at the meeting of the Society of American Bacteriologists, held at Philadelphia, in December, 1914.

To avoid any objection arising as to the possible existence of mixed cultures, or of "weak members" at the start, each strain of *B. coli* was plated, a single colony selected, and replated, and from this latter plate, several cultures were selected, inoculated on agar slants and after twenty-four hours cultivation, carried through on all media. The strain that showed the greatest amount of enzyme formation was selected as the "strongest" and the one to be subjected to experimentation.

In general, the technique of these experiments was identical with that already described, observations being made with special reference to indol production which was taken as the

index of proteolytic activities. To control the possible effect of the acid production of *B. coli*, upon proteolysis, three of the sugars, namely, glucose, lactose, and saccharose, had 1 per cent of calcium carbonate added to neutralize any acid formed. Another control was also carried along, a stock strain of *Sp. cholerae*, which produces but a slight amount of acid as compared with *B. coli*, but, on the other hand, gives a marked amount of indol. Seven different sugars were used besides the three containing calcium carbonate, making a total of ten inoculations for each organism. The organisms were cultivated in the respective sugar peptone solutions for six days, at which time small amounts of the cultures were poured over into fresh media for the continuance of the experiment, and then the Salkowski's test was applied to the original culture. After the seventh inoculation, when all the strains gave negative indol tests, experiments for reversions were begun, by transfering a small quantity of each of the cultures into plain peptone, and proceeding with the test as before.

TABLE X

Showing the effect of various carbohydrates upon the proteolytic activities of seven different strains of B. coli, and one of Sp. cholerae, as evidenced by the indol test

SUBCULTURE	CONTROL			GLUCOSE									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1	+	tr.	+	—	—	—	—	—	—	—	—	—	—
B. coli No. 2	++	++	++	tr.	tr.	—	tr.	—	—	—	—	—	—
B. coli No. 3	+++	++	+++	—	tr.	—	tr.	—	—	—	—	—	—
B. coli No. 4	++	++	++	tr.	tr.	—	tr.	—	—	—	—	—	—
B. coli No. 5	+++	+++	+++	tr.	tr.	—	tr.	—	—	—	—	—	—
B. coli No. 6	+++	+++	++	—	tr.	—	tr.	—	—	—	—	—	—
B. coli No. 7	++	++	++	tr.	—	—	tr.	—	—	—	—	—	—
Sp. cholerae	+++++	++++	+++++	tr.	—	—	tr.	—	—	—	—	—	—

TABLE XI.

Results of the indol tests in the experiments on reversion. * = *Positive test obtained with H₂SO₄ only.* † = *Positive test obtained only after the addition of nitrite*

SUBCULTURE				1	2	3	4	5	6	7	8	9	10
B. coli No. 1				—	—	—	—	—	sl. tr.	sl. tr.	tr.	—	+
B. coli No. 2				—	—	?	?	—	—	—	—	—	—
B. coli No. 3				—	—	?	—	?	—	—	?	—	—
B. coli No. 4				—	—	—	—	?	—	—	—	—	?
B. coli No. 5				—	—	—	—	?	—	—	?	tr.	?
B. coli No. 6				—	—	—	—	?	—	—	—	?	?
B. coli No. 7				—	—	sl. tr.	sl. tr.	—	—	—	sl. tr.	—	—
Sp. cholerae				—	—	?	—	sl. tr.	tr.	tr.†	+	+	+

TABLE X—*Continued*

SUBCULTURE	CONTROL			GLUCOSE + Ca₂CO₃									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1......	+	tr.	+	tr.	—	—	—	—	—	—	—	—	—
B. coli No. 2......	++	++	++	tr.	?	?	tr.	—	—	—	—	—	—
B. coli No. 3......	+++	++	+++	tr.	tr.	?	tr.	—	—	—	—	—	—
B. coli No. 4......	++	++	++	tr.	tr.	—	—	—	—	—	—	—	—
B. coli No. 5......	+++	+++	+++	tr.	tr.	?	tr.	—	—	—	—	—	—
B. coli No. 6......	+++	+++	++	tr.	?	?	tr.	—	—	—	—	—	—
B. coli No. 7......	++	++	++	tr.	tr.	?	tr.	—	—	—	—	—	—
B. cholerae.......	+++++	++++	+++++	+	tr.	—	—	—	—	—	—	—	—

TABLE XI—*Continued*
Reversion from above

				1	2	3	4	5	6	7	8	9	10
B. coli No. 1......	.			—	—	sl. tr.	—	tr.	—	tr.*	tr.*	—	tr.*
B. coli No. 2......				—	—	—	—	tr.	—	—	—	—	—
B. coli No. 3......				—	—	sl. tr.	++	++	—	—	—	—	—
B. coli No. 4......				—	—	tr.	+++	++	—	—	—	—	—
B. coli No. 5......				—	—	?	++	++	—	—	—		
B. coli No. 6......				—	—	?	++	++	—	—	—	—	—
B. coli No. 7......				—	—	tr.	++	++	—	—	—	—	—
B. cholerae.......				—	—	—	—	sl. tr.	—	tr.*	tr.*	tr.*	tr.*

TABLE X—*Continued*

SUBCULTURE	CONTROL			LACTOSE									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1......	+	tr.	+	—	—	—	—	—	—	—	—	—	—
B. coli No. 2......	++	++	++	tr.	tr.	?	tr.	—	—	—	—	—	—
B. coli No. 3......	+++	++	+++	+	tr.	tr.	tr.	—	—	—	—	—	—
B. coli No. 4......	++	++	++	tr.	tr.	—	—	—	—	—	—	—	—
B. coli No. 5......	+++	+++	+++	tr.	tr.	—	—	—	—	—	—	—	—
B. coli No. 6......	+++	+++	++	tr.	tr.	—	—	—	—	—	—	—	—
B. coli No. 7......	++	++	++	tr.	—	?	tr.	—	—	—	—	—	—
B. cholerae.......	+++++	++++	+++++	++	—	tr.	tr.	—	—	—	—	—	—

TABLE XI—*Continued*
Reversion from above

				1	2	3	4	5	6	7	8	9	10
B. coli No. 1......				—	—	—	tr.	sl. tr.	tr.	+*	+*	++	tr.
B. coli No. 2......				—	—	tr.	+	sl. tr.	tr.	+*	tr.*	+	+
B. coli No. 3......				—	—	sl. tr.	+	+	—	+*	+		
B. coli No. 4......				—	—	sl. tr.	+	++	+	+			
B. coli No. 5......				—	—	?	+	++	+	++			
B. coli No. 6......				—	—	?	+	+	+	+			
B. coli No. 7......				—	—	sl. tr.	+	+	+	+			
Sp. cholerae......				—	—	sl. tr.	+	+	+	+†	tr.†	+	tr.

TABLE X—*Continued*

SUBCULTURE	CONTROL			LACTOSE + $CaCO_3$									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1......	+	tr.	+	−	−	−	−	−	−	−	−	−	−
B. coli No. 2......	++	++	++	tr.	tr.	?	tr.	−	−	−	−	−	−
B. coli No. 3......	+++	++	+++	tr.	−	?	tr.	−	−	−	−	−	−
B. coli No. 4......	++	++	++	tr.	tr.	−	−	−	−	−	−	−	−
B. coli No. 5......	+++	+++	+++	tr.	?	−	tr.	−	−	−	−	−	−
B. coli No. 6......	+++	+++	++	tr.	?	?	tr.	−	−	−	−	−	−
B. coli No. 7......	++	++	++	tr.	tr.	−	−	−	−	−	−	−	−
Sp. cholerae......	++++	++++	+++++	+	tr.	+	tr.	tr.	−	−	−	−	−

TABLE XI—*Continued*
Reversion from above

SUBCULTURE				1	2	3	4	5	6	7	8	9	10
B. coli No. 1......				−	−	?	−	−	−	−	−	−	−
B. coli No. 2......				−	−	sl. tr.	sl. tr.	sl. tr.	tr.	+*	+*	+	+
B. coli No. 3......				−	−	tr.	+	++	+				
B. coli No. 4......				−	−	?	+	++	+				
B. coli No. 5......				−	−	sl. tr.	+	+	+				
B. coli No. 6......				−	−	−	+	+	+				
B. coli No. 7......				−	−	sl. tr.	+	+	+				
Sp. cholerae......				−	−	sl. tr.	tr.	+	+	tr.	sl. tr.	tr.	+

TABLE X—*Continued*

SUBCULTURE	CONTROL			SACCHAROSE																	
	1	2	3	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
B. coli No. 1.	+	tr.	+	tr.	−	tr.	−	−	−	−	−	−									
B. coli No. 2.	++	++	++	tr.	tr.	−	tr.	−	−	−	−	−									
B. coli No. 3.	+++	++	+++	++	+	+	+	+	+	tr.	+	+	+	+	+	tr.	+	+	tr.	sl. tr.	sl. tr.
B. coli No. 4.	++	++	++	tr.	tr.	−	tr.	−	−	−	−	−									
B. coli No. 5.	+++	+++	+++	++	+	+	+	+	+	tr.	+	+	+	+	+	++	tr.	+	tr.	+	+
B. coli No. 6.	+++	+++	++	++	+	+	+	+	+	+	+	+	tr.	+	++	tr.	tr.	+	tr.	+	tr.
B. coli No. 7.	++	++	++	tr.	−	−	tr.	−	−	−	−	−									
Sp. cholerae..	++++	++++	+++++	+	tr.	−	tr.	−	−	−	−	−									

TABLE XI—*Continued*
Reversion from above

SUBCULTURE				1	2	3	4	5	6	7	8	9	10								
B. coli No. 1...				−	−	−	−	−	−	−	−	tr.	sl. tr.								
B. coli No. 2...				−	−	−	−	tr.	+*	tr*	+*	tr.	tr.								
B. coli No. 3...				±																	
B. coli No. 4...				−	−	+	++	++	+												
B. coli No. 5...				±																	
B. coli No. 6...				±																	
B. coli No. 7...				−	−	+	++	+	+												
Sp. cholerae...				−	−	+†	tr.	sl. tr.	sl. tr.	tr.†	tr.†	tr.									

TABLE X—*Continued*

SUBCULTURE	CONTROL			SACCHAROSE + CaCO₂											
	1	2	3	1	2	3	4	5	6	7	8	9	10	11	12
B. coli No. 1....	+	tr.	+	tr.	—	tr.	—	—	—	—	—	—	—	—	—
B. coli No. 2....	++	++	++	—	tr.	—	tr.	—	—	—	—	—	—	—	—
B. coli No. 3....	+++	++	+++	+	+	+	+	+	+	tr.	+	+	tr.	+	tr.
B. coli No. 4....	++	++	++	tr.	tr.	?	tr.	+	—	—	—	—	—	—	—
B. coli No. 5....	+++	+++	+++	+	+	?	+	+	+	+	tr.	+	tr.	tr.	tr.
B. coli No. 6....	+++	+++	++	+	+	+	+	+	+	+	+	tr.	+	+	+
B. coli No. 7....	++	++	++	tr.	tr.	?	tr.	—	—	—	—	—	—	—	—
Sp. cholerae....	+++++	++++	+++++	+	tr.	tr.	tr.	tr.	—	—	—	—	—	—	—

TABLE XI—*Continued*
Reversion from above

				1	2	3	4	5	6	7	8	9	10	11	12
B. coli No. 1....				—	—	—	—	—	tr.*	—	—	tr.*	—	tr.*	—
B. coli No. 2....				—	—	sl.tr.	tr.	tr.	tr.*	tr.	+*	+*	+*	+*	+*
B. coli No. 3....				±											
B. coli No. 4....				+	+	+	+	+	+	+	+	tr.	+	+	+
B. coli No. 5. ..				—	—	—	?	tr.	tr.	+	+	tr.	+		
B. coli No. 6....				±											
B. coli No. 7....				—	—	tr.	+++	++	+	+					
Sp. cholerae....				—	—	—	sl. tr.	tr.	tr.	tr.†	tr.	+	tr.	+	+

TABLE X—*Continued*

SUBCULTURE	CONTROL			MALTOSE									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1......	+	tr.	+	tr.	—	—	—	—	—	—	—	—	—
B. coli No. 2......	++	++	++	tr.	tr.	?	?	?	—	—	—	—	—
B. coli No. 3......	+++	++	+++	tr.	tr.	?	—	—	—	—	—	—	—
B. coli No. 4......	++	++	++	—	tr.	?	?	?	—	—	—	—	—
B. coli No. 5......	+++	+++	+++	tr.	tr.	?	?	—	—	—	—	—	—
B. coli No. 6......	+++	+++	++	tr.	tr.	?	?	—	—	—	—	—	—
B. coli No. 7......	++	++	++	tr.	tr.	?	?	—	—	—	—	—	—
Sp. cholerae......	+++++	++++	+++++	+	tr.	?	?	—	—	—	—	—	—

TABLE XI—*Continued*
Reversion from above

				1	2	3	4	5	6	7	8	9	10
B. coli No. 1......				—	—	sl. tr.	—	—	sl. tr.	sl. tr*	—	tr.	tr.
B. coli No. 2......				—	—	sl. tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
B. coli No. 3......				tr.	—	tr.	+	++	+				
B. coli No. 4......				—	—	+	+	+	+				
B. coli No. 5......				—	—	+	++	+	+				
B. coli No. 6......				—	—	+	++	+	+				
B. coli No. 7......				—	tr.	+	++	+	+				
Sp. cholerae......				—	—	—	tr.	tr.	tr.	tr.†	tr.†	tr.	tr.

TABLE X—*Continued*

SUBCULTURE	CONTROL			GALACTOSE									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1......	+	tr.	+	tr.	—	—	—	—	—	—	—	—	—
B. coli No. 2......	++	++	++	—	tr.	—	—	—	—	—	—	—	—
B. coli No. 3......	+++	++	+++	tr.	?	—	—	—	—	—	—	—	—
B. coli No. 4......	++	++	++	tr.	?	—	—	—	—	—	—	—	—
B. coli No. 5......	+++	+++	+++	tr.	?	—	—	—	—	—	—	—	—
B. coli No. 6......	+++	+++	++	tr.	?	—	—	—	—	—	—	—	—
B. coli No. 7......	++	++	++	tr.	?	—	—	—	—	—	—	—	—
Sp. cholerae......	++++	++++	+++++	—	?	—	—	—	—	—	—	—	—

TABLE XI—*Continued*
Reversion from above

SUBCULTURE				1	2	3	4	5	6	7	8	9	10
B. coli No. 1......				—	—	sl. tr.	+	+	+	tr.	tr.	++	+
B. coli No. 2......				—	—	—	—	tr.	tr.	—	tr.	sl. tr.	
B. coli No. 3......				—	—	sl. tr	+	+	+				
B. coli No. 4......				—	—	sl. tr.	++	+	+				
B. coli No. 5......				—	—	?	+	+	+				
B. coli No. 6......				—	—	+	+	+	+				
B. coli No. 7......				—	—	tr.	+	+	+				
Sp. cholerae......				—	—	—	—	—	+	tr.	tr.	tr.	tr.

TABLE X—*Continued*

SUBCULTURE	CONTROL			DEXTRIN									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1......	+	tr.	+	—	—	—	—	—	—	—	—	—	—
B. coli No. 2......	++	++	++	tr.	tr.	—	tr.	—	—	—	—	—	—
B. coli No. 3......	+++	++	+++	tr.	tr.	—	tr.	—	—	—	—	—	—
B. coli No. 4......	++	++	++	tr.	tr.	—	—	—	—	—	—	—	—
B. coli No. 5......	+++	+++	+++	tr.	—	—	—	—	—	—	—	—	—
B. coli No. 6......	+++	+++	++	tr.	—	—	—	—	—	—	—	—	—
B. coli No. 7......	++	++	++	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	?
Sp. cholerae......	++++	++++	+++++	tr.	—	—	tr.	—	—	—	—	—	—

TABLE XI—*Continued*
Reversion from above

SUBCULTURE				1	2	3	4	5	6	7	8	9	10
B. coli No. 1......				—	—	—	—	+*	++*	+*	+*	++*	+
B. coli No. 2......				—	—	tr.	—	sl. tr.	tr.	+*	tr.*	+	+
B. coli No. 3......				+	+	+	++	+	+				
B. coli No. 4......				+	+	+	+	+	+				
B. coli No. 5......				+	+	+	++	+	+				
B. coli No. 6......				+	+	+	++	++	+				
B. coli No. 7......				+	+	+	++	+	+				
Sp. cholerae......				—	—	sl. tr.	sl. tr.	sl. tr.	tr.	tr.	tr.†	+	tr.

TABLE X—*Continued*

SUBCULTURE	CONTROL			MANNITE									
	1	2	3	1	2	3	4	5	6	7	8	9	10
B. coli No. 1....	+	tr.	+	tr.	—	—	—	—	—	—	—	—	—
B. coli No. 2....	++	++	++	tr.	tr.	?	—	—	—	—	—	—	—
B. coli No. 3....	+++	++	+++	tr.	tr.	?	—	—	—	—	—	—	—
B. coli No. 4....	++	++	++	tr.	tr.	?	—	—	—	—	—	—	—
B. coli No. 5....	+++	+++	+++	tr.	tr.	?	—	—	—	—	—	—	—
B. coli No. 6....	+++	+++	++	tr.	tr.	?	—	—	—	—	—	—	—
B. coli No. 7....	++	++	++	tr.	tr.	?	—	—	—	—	—	—	—
Sp. cholerae....	+++++	++++	+++++	+	tr.	?	—	—	—	—	—	—	—

TABLE XI—*Continued*
Reversion from above

				1	2	3	4	5	6	7	8	9	10
B. coli No. 1....				—	—	—	—	—	—	—	tr.	—	—
B. coli No. 2....				—	—	+	+	+	+	tr.*	++	+	+
B. coli No. 3....				—	—	+	+	+	+				
B. coli No. 4....				—	—	+	+	+	+				
B. coli No. 5....				—	—	sl. tr.	+	+	+				
B. coli No. 6....				—	—	+	+	+	+				
B. coli No. 7....				—	—	tr.	+	+	+				
Sp. cholerae....				+†	tr.†	+++†	++†	+++†	+†	+	+†	+†	+†

A study of the accompanying tables will best convey to the reader the results obtained. It will be noted that, strain no. 1 is the "weakest" member of the group, giving but little indol in the controls and but traces in five of the ten sugar media used. Strains 2, 4 and 7 are readily changed by all sugars, with a rapid disappearance of indol formation. The most interesting results, however, were seen in strains 3, 5 and 6. These were affected similarly to nos. 2, 4 and 7 by all the sugars except saccharose, in which medium the indol reaction continued in each case until the 18th transfer, at which time the experiment was discontinued. It was suspected that these latter were strains of *B. coli communis*, and since *B. communis* normally does not ferment saccharose with the production of gas, possibly not affected by this carbohydrate. Inoculating the original agar culture of nos. 3, 5 and 6 into saccharose media proved this supposition to be correct. At the time of the seventh transfer, the amount of indol produced by these three strains was markedly less than at the beginning of the experiment. This, coupled with the fact that the bacteria were

subjected to saccharose for over forty days, suggested the possibility that they had acquired the function of fermenting saccharose, and that the resultant glucose, in its turn, had its effect upon the indol production as evidenced in the other experiments. Planting these organisms after the seventh subculture into saccharose agar proved this to be the case, as each of the strains, 3, 5 and 6, now fermented saccharose with the production of gas to a moderate degree.

The writer desires to emphasize at this juncture what appears to him a certainty, namely, that it is the carbohydrate per se that causes the fluctuating biological modification noted in those experiments, though a definite *modus operandi* is not known. This is well seen in the action of saccharose which had but slight effect upon three of seven strains of *B. coli* until the organisms were capable of splitting it into glucose, which, in its turn, effected the amount of indol produced. The fact that the inhibition was not complete, does not contradict this supposition, for it can be accounted for either by the presence of individual bacteria that remained unaltered by virtue of their greater resistance, or by the fact that the attack on saccharose was sufficiently slow to permit a small amount of proteolytic cleavage. Further proof of such effects of carbohydrates will be offered below.

The cholera spirillum, though it produced a large amount of indol in the control, readily succumbed to the action of the various carbohydrates, and, in several instances, even sooner than the *B. coli*. The fact that this strain of cholera spirillum produced but very little acid would indicate that the inhibitory action was not due to acid accumulation. The possible effect of excess acid is likewise shown to be negligible by the results obtained in the carbohydrate media, wherein, as may be seen by consulting the tables, the differences were so slight that they cannot be regarded as having any bearing.

It is of interest to note the differences in the action of the various carbohydrates. Saccharose has already been commented upon. Galactose, it appears, had the most pronounced effect upon all of the organisms, permitting but a single trace of indol

in five of the eight organisms in the first culture tube, and practically none thereafter. Dextrin, on the other hand, showed the greatest variation in its effect upon the different strains of *B. coli*. Strain no. 1 gave no indol throughout the experiment; both nos. 5 and 6 gave a single trace in the first culture but none thereafter, whereas no. 7 gave a trace in each sub-culture up to the tenth, at which time the experiment was discontinued. Maltose and mannite appear to have a more pronounced inhibitory effect upon indol formation than either glucose, lactose or saccharose and would stand intermediate between them and galactose.

Experiments were undertaken to determine the permanency of this change in biological activities using the same technique as previously described. The bacteria, after being carried through the carbohydrate peptone media for at least seven transfers, were then inoculated into plain peptone, making successive transfers until three consecutive positive indol tests were obtained.

By referring to Table XI, the most striking difference is seen between the action of glucose and dextrin. In the case of glucose the indol reaction reappeared only in the cases of *B. coli* nos. 1, 2, 5 and 7, and the cholera spirillum, the others remaining negative up to the tenth transfer in plain peptone, at which time the experiment was discontinued. For some unexplained reason, the re-appearance of the property of indol formation was slower and less marked in this set of experiments than on previous occasions. When contrasted with the action of dextrin, a marked difference is seen. This substance permits a prompt return of the indol producing property in the first transfer of the different strains of bacteria used, with the exception of *B. coli* nos. 1 and 2, and the cholera spirillum. Inasmuch as this peculiarity was evident in each of the sugars, it would appear that it was not due to the carbohydrate, but to a possible greater susceptibility on the part of these strains of bacteria. *B. coli* nos. 3, 4, 5, 6 and 7 gave positive indol tests on the third or fourth transfers quite uniformly in all sugars except glucose as already mentioned. It may be concluded that dextrin acts entirely unlike the other carbohydrates, having, in

the first place, a somewhat selective action on the different strains of *B. coli*, and secondly, permitting an immediate return to normal proteolytic activity, under the conditions of experimentation here outlined.

Aside from the peculiar manner in which *B. coli* nos. 1 and 2 and the cholera spirillum acted on reversion in contrast to the other strains, there is another observation worthy of mention. It was noted that frequently in doing the Salkowski test with these colon strains, a positive reaction occurred without the addition of nitrite. This is rarely found with the *B. coli* and had not been noticed in any of the control cultures nor in the previous experiments. This peculiarity of the test was quite irregular in its occurrence, it appeared as if at will, and disappeared and re-appeared without any apparent reason, since the conditions of the experiment were unaltered. The reverse was often noted with the cholera spirillum, in which case, instead of obtaining a positive indol test on the addition of acid alone, none occurred until nitrite was added. This observation would indicate some changes in the power of nitrite formation entirely independent of that of indol production, and would well fit in with the carbohydrate effect upon the other biological activities already mentioned.

In order further to ascertain the action of the various sugars on the biological activities of the different strains of bacteria in question, a proteid-free medium was used as a base, to which 3 per cent of the different sugars was added. Those used were glucose, lactose, saccharose, maltose, and dextrin. Inoculations were made from the same stock cultures of *B. coli* as in the above experiments, a control of each strain being carried in the proteid-free medium itself. The medium selected was the writer's modification of Naegeli's proteid-free medium and had the following composition:

Ammonium tartrate	10.0
Potassium phosphate	1.0
Magnesium sulphate	0.2
Calcium chloride	0.12
Glycerin	20.0
Water	1000.0

The medium base was made up in moderate quantities, sterilized in the autoclave and kept until needed. The sugars were added to separate small quantities as required, tubed and sterilized by the intermittent method. Inoculations were made every fourth or fifth day over a period of fifteen weeks, making a total of twenty-six transfers. Tests were made from the last culture tube to determine biological variations by planting into the various laboratory media, final observations being noted after seven days inoculation.

This series of experiments was not as successful as the writer desired. Many of the strains suddenly refused to grow, and were lost at different stages of the experiment. This can be explained as due either to a lack of proper nutrition, or, possibly, to an inhibitory action of the sugars, or both. "Sudden death" was also noted by Peckham (1897) in her experiments with *B. coli* grown in glucose peptone, and is accounted for by her as due to the accumulation of by-products coupled with the complete exhaustion of the bacteria. She believes that on account of incomplete proteolytic activities, through the preference for the carbohydrate food, there is a deficiency in plastic material of the bacteria sufficient to interfere with reproduction and building up of the cells. Besides the loss of a number of strains, several were found to be contaminated and had to be discarded.

Though but five cultures were subjected to the carbohydrate action, and two controls were carried through to the completion of the experiment, the writer believes the results worthy of record, particularly since they verify the results obtained in the work above reported. This is also desirable since the intent of these experiments was to show the ability of the controls to retain their various biological characteristics even though subjected to carbohydrates.[2]

Table XII indicates the results obtained. Strains 3 and 6, subjected to the action of glucose in a proteid-free medium, seem to compare well with the results obtained with glucose

[2] These experiments are being repeated, and will again be reported on at a later date.

TABLE XII

Action of carbohydrates in a peptone free medium upon the cultural characteristics of several strains of *B. coli*—marked inhibition by glucose, lactose and maltose, in strain No. 3, and only slight by glucose and saccharose in strain No. 6

STRAIN NO.	POTATO		MILK		GLUCOSE		LACTOSE		SACCHAROSE		MALTOSE		DEXTRIN		MANNITE		FERMENTATION TUBE		INDOL
	Br. Gr.	Disc.	Acid	Coag.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	
Control..... 3	++	+++	+++	+++	+++	++	++	++	++	–	+++	++	+++	++	+++	++	++	+++	+
Glucose..... 3	–	–	+	–	+++	–	++	–	+	++	++	–	–	–	++	–	+	–	–
Glucose..... 6	++	++	+++	+++	–	++	–	++	++	+	+++	++	++	++	–	++	–	++	–
Lactose..... 2	–	–	++	–	+++	++	+++	++	++	+	+++	+	++	+	+++	+++	–	–	–
Saccharose . 5	++	++	+++	++	+++	++	+++	+++	++	–	+++	++	++	++	++	++	+++	+++	Sl
Control..... 1	+++	++	+++	+++	+	–	–	+++	++	–	+++	++	++	++	++	++	+++	+++	–
Maltose..... 1	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–

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broth media. *B. coli* no. 3 is markedly changed from its control, which itself appears weaker in its cultural characteristics than controls carried in broth. The changes in the other strains need but little comment. When compared with the glucose action as seen in the previous tables, a similar action may be noted here.

Too much stress cannot be placed upon so few experiments with proteid-free media, but the writer feels confident that inhibitory action of other sugars than glucose does occur, and can readily be demonstrated. It may be well in passing, to note that indol production is invariably completely suppressed. Next to indol, the greatest amount of inhibition is manifest upon gas production, then, on lab enzyme and the characteristic growth on potato and finally on acid production. This sequence is not always adhered to, but holds good in a general way. All of these activities seem to correspond very well indeed with the action of glucose in broth media.

Peckham in a series of experiments along similar lines prefers to regard the changes as not due to any direct carbohydrate, or chemical effect, but rather to what she terms exhaustion, sheer inability to produce normal biological effects on account of previously expended energy. She describes numerous experiments carried out with a number of strains of *B. coli*, in which she claims that proteolytic activities were suspended when these organisms were grown in fresh peptone sugar broth. The index she took for determining proteolysis was the amount of indol produced by those subjected to carbohydrate as compared to control cultures. She concluded that this suppression of proteolytic activities was due to the preference of the *B. coli* for carbohydrate, a more readily assimilated food, to proteins, with a subsequent exhaustion on the part of the bacteria by the time they reached the protein material. That this "exhaustion of energy" was not due to the amount of lactic acid produced was proven by her in another series of tests in which quantities of lactic acid were added to similar peptone solutions without carbohydrate, and in which indol was produced as in the control cultures.

This preference for carbohydrate food to the exclusion of proteolysis has also been shown by other investigators to occur both with *B. coli* and numerous other bacteria, but in the opinion of the writer there is still an open question in regard to the *modus operandi*, namely, whether it is due to sheer exhaustion, or to a certain yet unexplained carbohydrate effect upon the metabolic activities of the bacteria.

Peckham did not return the *B. coli* into plain peptone to determine whether the absence of indol was due to a simple suspension or inhibition of proteolytic activities, or whether this inhibition was a pronounced one extending over a period of time. Had she done so, she would probably have found, as did the writer, that this inhibition was a decided one, not readily overcome, that it varied with different strains of *B. coli*, and also varied with the carbohydrate used. In the light of the experiments here reported the writer feels quite convinced that exhaustion, though it may be given a certain amount of credit, cannot explain all of his findings. This contention is emphasized by: (1) The different effects obtained on different strains of *B. coli*. (2) Differences in effect by the various sugars, dextrin acting much more readily, and permitting an immediate return of indol formation, whereas in the case of glucose, the carbohydrate effect was more gradual and more lasting. (3) The fact that in an occasional experiment, the organism would not revert to its original type, but remain permanently changed in some or even all of its biological characteristics. (4) Differences in the quantity, and time of disappearance or reappearance of the various enzyme activities, with no special sequence of events. (5) Exhaustion, though in spite of the above factors it might still be regarded as being the cause in regard to indol, cannot explain the more gradual loss of lab enzyme, fermentation of carbohydrates with the production of gas, the lack of typical growth on potato and finally loss of acid production. If we were still to insist that it is a matter of exhaustion, we must assume that it was so profound that it could have been handed down from one culture to another over certain periods of time as evidenced in the experiments for reversion. This would

be contrary to any existing ideas in respect to the effects of exhaustion and would intimate that exhaustion can be inherited.

The following conclusions may be drawn from the foregoing experiments with *B. coli*.

1. Both glucose and phenol give rise to either partial or complete inhibition of the cultural characteristics of some strains of *B. coli*. Sodium chloride and sodium sulphate also display inhibitory action but by no means as marked as either of the above compounds.

2. When complete, the change is more lasting, but there is always present a strong tendency for the modified bacteria to return to their former status of biological activities.

3. Occasionally, complete reversion does not take place, in which case the organism remains permanently devoid of certain enzymes or of the power of fermenting one or more of the carbohydrates.

4. There is no well defined nor constant sequence of events either during the process of modification or reversion, and no relationship between the changes produced in the various enzymes, but, it may be stated, in a general way, that indol production is the first to disappear, then, the fermentations of the various carbohydrates, the characteristic growth on potato, lab enzyme, and finally, acid production.

In closing, the writer desires to take this opportunity of expressing his sincerest thanks to his students, Messrs. Bingaman, Braude, Denehey, Nachamofsky, Rubinsky, Russo, and Miss Wright, for their kind coöperation in the above work.

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A NEW CULTURE MEDIUM FOR THE TUBERCLE BACILLUS

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Contamination is an occurrence which has always troubled bacteriologists in the isolation and cultivation of the tubercle bacillus. Even the various digestive methods which are used at present, such as the antiformin, the sodium carbonate, and the sodium hydroxid methods, have failed to yield uniformly satisfactory results. It is obvious that the ideal culture medium for this purpose would be one which included some substance that had the property of preventing the growth of other microorganisms while not interfering with the isolation and reproduction of the tubercle bacillus.

The observation of v. Drigalski and Conradi (1902), that crystal violet was able to inhibit the growth of many bacteria but had no effect on the cultivation of typhoid and colon bacilli was the first step in this direction. Then followed the splendid researches of Churchman (1912) who worked with a number of common dyes and discovered that gentian violet possessed a striking selective power. He found that by making divided plates, one-half filled with plain agar and the other filled with agar to which 0.001 per cent gentian violet was added, and stroking the surface with a mixed culture, he could get rid of the contaminating organisms. For instance, by this means he purified a culture of *B. tuberculosis* which had become contaminated with *B. subtilis*. The former organism is "gentian-negative" while the latter, like almost all air contaminations, is "gentian-positive." He states that the effect of gentian violet on gentian-positive organisms is better described as bacteriostatic, rather than bactericidal, meaning that the dye suspends repro-

duction without implying that the organisms are necessarily killed.   He finds that this gentian reaction is much more definite and constant than the Gram stain.

Next came the attempt of Petroff (1915) to devise a simple, practical and reliable method for the isolation and cultivation of the tubercle bacillus from the sputum and feces.   He experimented with gentian violet, methyl violet, methylene blue, crystal violet, and fuchsin added to a meat-juice-glycerin -egg medium.   He found that gentian violet was the most favorable stain on account of its inhibitory action on many organisms and reported that he obtained sixty-nine positive cultures from sixty-nine specimens of sputum from practically all stages of tuberculosis.   Six of these specimens were negative by direct microscopic examination.

Shortly after the publication of Petroff's article, we began the use of his method.   We were soon impressed with the inhibitory effect of the sodium hydroxid and gentian violet which he used, but not with the medium as a whole.   It did not seem to contain enough moisture to prevent rapid drying; it required inspissation on three successive days, with the result that frequently an uneven surface was obtained in spite of the utmost care; also, it occasionally became contaminated with spore bearing organisms which would usually cause liquefaction of the medium with consequent destruction of hopeful cultures.

It seemed to us that if a gentian violet medium could be made which did not require inspissation, gave a smooth surface, contained sufficient moisture, and could be sterilized in an autoclave, we should have a nearly ideal culture medium.

While working with the medium devised by Besredka (1913) for growing tubercle bacilli to make his tuberculin, which serves as a good antigen in tuberculosis complement fixation tests, it occurred to us to utilize it in an attempt to overcome the defects of Petroff's medium.   We believe we have accomplished this by making a medium as here described.

PREPARATION OF THE MEDIUM

1. Egg-white solution. This is made by diluting the egg-white with ten parts of distilled water and thoroughly shaking. The fluid is opalescent and contains numerous whitish flakes. To clear it, it is passed through a thin layer of cotton and then heated to 100°C. to hasten precipitation. It is then filtered through paper.

2. Egg-yolk solution. The yolks are diluted with ten parts of water and well stirred. The very cloudy emulsion is clarified by adding normal sodium hydroxid. Too much hydroxid is harmful and therefore complete solution of the yolk is not desirable. The emulsion should be slightly turbid. To attain the proper degree of turbidity, one cubic centimeter of normal sodium hydroxid is usually added to each one hundred cubic centimeters of the emulsion. This is not a constant amount, however, because some yolks will be completely dissolved by less than half this amount of alkali. The solution is heated to 100°C. and filtered.

3. Meat infusion. Five hundred grams of finely chopped lean veal are covered with one liter of water containing 15 per cent of glycerin, allowed to infuse for twenty-four hours and filtered; 5 grams of sodium chlorid are added, and the infusion heated to boiling. It is again filtered and then rendered plus 1 per cent alkaline.

With the above solutions, the medium is made as follows: Place 300 cc. of the 10 per cent egg-white solution in a liter flask; 300 cc. of the 10 per cent egg-yolk solution in another flask; and 400 cc. of the meat infusion, to which is added 15 grams of powdered agar-agar, in a third flask. These are then sterilized in the autoclave at 15 pounds pressure for fifteen minutes. They are removed from the sterilizer and, while hot, 1 cc. of a 1 per cent alcoholic solution of gentian violet is added to the broth-agar. The contents of this flask are now poured into that containing the egg-white and then the egg-yolk is added. The whole is poured back and forth from this flask to another so as to insure thorough mixing and then it is tubed and slanted.

The tubes are left in their slanted position for about seventy-two hours at room temperature until the contents are well set. The cotton plugs are then trimmed and flamed and the tubes sealed with corks. This medium presents the same smooth inoculating surface as ordinary agar slants, contains as much moisture, is quickly made and is rendered absolutely sterile.

METHOD OF ISOLATING TUBERCLE BACILLI FROM SPUTUM

About 10 cc. of fresh sputum, which has been thoroughly washed in a running stream of sterile 0.85 per cent salt solution, is placed in a sufficiently large centrifuge tube containing a piece of blue litmus paper. An equal amount of 3 per cent sodium hydroxid is added and the whole well shaken. It is put in the incubator for about one hour or until the sputum is fairly well digested. The mixture is neutralized with normal hydrochloric acid, then centrifugalized, and after removing the supernatant fluid the sediment is planted on several tubes containing the herewith described medium by means of a large platinum loop or a capillary pipette. After from five to fourteen days incubation, a good growth appears which is free of any contamination.

This method has given us uniformly good results and the medium remains serviceable for at least one month.

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BACILLUS ABORTUS (BANG) AS AN ETIOLOGICAL FACTOR IN INFECTIOUS ABORTION IN SWINE

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So far as we are able to learn from the literature, the cause of infectious abortion in swine has never been determined. Lynch in his "Diseases of Swine," says, "Infectious abortion results from the infection of the genital passages by some specific germ, the true nature of which is as yet undetermined. The disease is not nearly so common as in mares and cows, and, while it may run through an entire herd, it is not likely to be spread from one farm to another except in unusual instances." He further states, "The infectious type of the disease is especially mild in its symptoms, and unless the animals are carefully watched the pigs may be slipped without any notice of the fact until several weeks later, when it is found that the sow is no longer pregnant." He also says, "The nearer to full term the sow is at the time of abortion, the less dangerous the occurrence and the more mild the symptoms. Signs of threatening abortion are loss of appetite, restlessness, making of the bed, shivering, trembling of the muscles, dulness, and in some cases very severe labor-pains."

While considerable work has been done with regard to infectious abortion in mares and cows in this laboratory, only three outbreaks of infectious abortion among sows, have come to our investigation. We have found that it is no uncommon occurrence for one or two sows in a herd to abort. Numerous cases of this kind have been studied by the writers, with no etiological results, which would lead to the opinion that such abortions were due to some accident.

In the large outbreak investigated by the writers early this year, some twenty sows aborted in rather quick succession. On visiting the place, it was found that one sow had aborted the night before, and two of the fetuses together with the attached afterbirth were brought to the laboratory for examination. The fetuses were fairly well developed, although not far enough along to have any hair. On one of the afterbirths there were noted numerous brownish, villus-like projections ranging in size from 1 to 3 mm. in diameter. They were so numerous in some places as to become confluent. On opening these, a dark serous fluid was noted. In our investigations upon the etiology of infectious abortion of animals, streak dilutions on agar or agar serum are always made and incubated under aerobic, Nowak, and anaerobic conditions. In this instance, streak dilutions were made on a series of agar plates, the agar being slightly alkaline to phenolphthalein, with material from the nodules on the afterbirth, from bits of the afterbirth from both pigs, and from the contents of the umbilicus, heart, liver, stomach and kidney of each fetus. From appearances, the kidneys of the pigs were very much enlarged and gorged with blood. As these pigs had lain on straw for a few hours, several of the plates showed on incubation in the air the growth of a considerable number of contaminating organisms, such as *B. coli*, *B. subtilis*, etc. This, of course is what would be expected on plating the afterbirth as the material had lain on straw for several hours before being secured by us. Cultures from the internal organs of the fetuses, however, were nearly sterile. The clear places on the plates were marked with India ink, after which they were subjected to the cultural method of Nowak.[1] The material was incubated at 37°C. for four days and removed from the jars. On casual examination of the petri dishes, we were led to believe that they contained nothing but the growth of bacteria usually encountered in plating material which had lain on the ground for some hours. Upon examining one of the plates carefully with a Coddington lens, however, there were noted in some of the clear spaces of the dishes a few very

[1] E. S. Good, Investigations of the Etiology of Infections Abortion of Cows and Mares, Bull. No. 165, Ky. Agri. Exper. Sta., 1912, p. 249.

small dew drop like colonies, which on being examined with the microscope resembled in every respect colonies of the *B. abortus* of Bang. Examined by the aid of the hand lens they were nearly water clear to direct light and of bluish tint to reflected light. They were round and raised, with exceptionally well defined borders. Most of these minute dew drop colonies were homogeneous, with the exception that a few of them had a few granules in the center, typical of many colonies produced by the *B. abortus*. Viewing the larger colonies under the microscope, it was seen that the centers had assumed a granular consistency, while the outer portion was homogeneous and transparent. The colonies on some of the plates were so large that they might have been taken for some other species, measuring $1\frac{3}{4}$ mm. in diameter. These conformed, however, to similar colonies of *B. abortus* derived from the tissues of the cow. To reflected light they had assumed an amber color, the centers having a whitish appearance. On examining stained preparations of these colonies, the morphology of the organism was identical, so far as we could determine, with *B. abortus*. On examining the plates carefully with a Coddington lens and microscope, colonies resembling those mentioned and measuring from a pinpoint to 1.5 mm. in diameter were seen on streaks made from the blood of the umbilicus, on the streaks made from the small nodule-like growths on one of the afterbirths, and from the afterbirth and internal organs of the fetuses. Eighty-four colonies in all were counted on streaks made from one of the small nodules. Some of these colonies were so small that they could not have been seen with the naked eye. There were one or two colonies on the streaks made from the livers. The plates streaked with the amniotic fluid were almost completely covered with contaminating bacteria, but, in the clear spaces five dew drop colonies were noted. The streaks of the contents of the stomachs of both pigs showed numerous small dew drop colonies. Streaks from the kidneys were negative. Stained preparations had been made from the different organs mentioned but were not examined until the cultures had developed. Upon examining these preparations, germs identical with the *Bacillus abortus* (Bang) were seen in large numbers in those made from the stomach contents of the pigs.

Typical colonies were streaked on agar slants, some of which were incubated by the Nowak method and others in the air. At the end of twenty-four hours, no visible growth was noted on the streak cultures incubated in the air. At the end of forty-eight hours, however, some little growth could be detected even with the naked eye, and at the end of seventy-two hours, quite a luxuriant growth of the organism was obtained, in appearance identical with streak cultures made with the *B. abortus*. On examining the tubes which had been incubated under the Nowak method, we found that they had made but slightly better growth than those incubated in the air. Streaks on agar at room temperature showed no growth in the time mentioned. We came to the conclusion that if this organism was the *B. abortus* and grew in the air after the first generation it was different from any we had ever isolated. The organism responded to the following tests in the following manner: It was found to be non-motile; gram negative; did not produce gas in either lactose or glucose; did not coagulate milk; grew readily in plain bouillon, showing a fair degree of cloudiness at the end of seventy-two hours; and did not liquefy gelatin. Serum-agar tubes heavily inoculated with this organism and quickly solidified in ice water and incubated in the air, developed a growth characteristic of the *B. abortus*, as noted by this laboratory, in that a narrow ring of growth appeared as a slight haze 3 mm. beneath the surface of the medium at the end of sixty hours, and eventually extended to the top of the medium. All the above tests conform to the biological and cultural characteristics of the Bang bacillus.

We were not, however, satisfied that the organism isolated was the *Bacillus abortus* on account of its growing in the air so readily, so we subjected the culture to the agglutination and complement fixation tests, using the serum from a rabbit made immune to the Bang bacillus, which agglutinated in high dilutions. It was found that this serum agglutinated our organism in a dilution of 1:1200, which was exactly the same dilution in which the serum agglutinated an antigen made of a well known strain of the *B. abortus*, which had been obtained from an aborting cow. Using as an antigen the organism isolated from the

sows and the immune serum mentioned, the complement was completely fixed with 0.02 cc. of serum.   We were thus convinced that this organism was identical with the germ that produces abortion in the cow, the only difference being that it grew readily in the air after the first generation, while the cultures we have derived from the cow usually do not grow in the air until after being cultured for several generations by the Nowak method. An exception to this rule, was discovered by Dr. Frank M. Surface,[2] who accidently inoculated a cow with a culture of the Bang bacillus which had repeatedly been transplanted for some two years from agar to agar, or from agar to plain peptone broth, and then back to agar.   He had obtained this particular strain while in Denmark.   The cow injected, aborted and Surface isolated the organism, the first generation of which developed in the air.   He was able to determine definitely, by using this organism as an antigen in the complement fixation test with an immune serum, that it was the Bang bacillus.   Surface states that the growth obtained in a Novy jar (Nowak method) was in no respect better than that obtained in the free air.

We cannot state whether the organism isolated from the aborting sow would have grown directly from the tissues, as we cultured none of the material in that way for any length of time.   Upon re-culturing the original material, which had been kept in the ice box, we found that the contaminating bacteria had become so numerous as to make streak dilutions impossible.

INOCULATION EXPERIMENTS WITH THE ORGANISM OBTAINED FROM THE ABORTING SOW

To test this organism further, a streak culture on an agar slant was washed with 5 cc. of physiological salt solution and 2 cc. of this material was diluted in 3 cc. of normal salt solution and injected intravenously into a pregnant sow, no. 1, on February 25, 1916.   On March 13, seventeen days after the inoculation, this sow aborted five fetuses.   The only symptom

[2] Surface, F. M., A Note on the Maintenance of Virulence by Bacillus Abortus Bang, Journal of Infectious Diseases, 1913, **12**, p. 359.

shown by this sow before aborting was that she did a great deal of rooting a day or two before slipping her pigs. As soon as she aborted she ceased rooting. The aborted fetuses, while quite well developed, were not haired over. Stained slides were made from the contents of the different organs. Streak dilutions were made of the heart, liver and stomach contents of each pig, as well as of the afterbirth, on 2 per cent agar poured in petri dishes and solidified. Some of the dishes were incubated in the air, while others were cultured according to Nowak. These fetuses were numbered 1 to 5. The organism with which this sow was injected was obtained from the heart, liver and stomach of pig no. 1; from the heart of pig no. 2; from the heart, liver and stomach of pig no. 3; from the heart and liver of pig no. 4; and from the heart and stomach of pig no. 5. Streak dilutions of these organs grown in the air showed no growth at the end of twenty-four hours. After forty-eight hours, however, the growth was distinctly visible to the naked eye, and at the end of seventy-two hours it was abundant. The streak dilutions grown under diminished oxygen (Nowak method) showed no more growth than that obtained in the air. Upon microscopical examination of the contents of the stomachs of pigs no. 1 to 5, it was seen that these organs harbored the germs in exceedingly large numbers.

On February 29, 1916, a pregnant sow (no. 2) was fed in ship-stuff the growth of the organism, obtained from the aborting sow, on two large agar slants washed off with 40 cc. of sterile normal salt solution. This sow was kept in an inclosure separate from sow no. 1. On March 10, 1916, she received the contents of five small agar tubes in a similar feed. On March 17, the attendant informed us that this sow was going to abort because she was acting like the other sow, previous to aborting, in vigorously rooting the ground floor of her pen. On March 19, nineteen days after being fed the initial dose of the organism, the sow aborted. We obtained three of the pigs. She had eaten the afterbirth, and in all probability had also eaten some of the pigs, as she had bitten out a large piece from the side of one of the pigs secured. After a long series of dilutions, we were able to isolate the original organism from the stomach contents

of one of these pigs. The bacillus was present in this instance in very small numbers.

On March 27, 1916, the tails of these sows were carefully washed, shaved and disinfected, the ends cut off, and 25 cc. of blood taken. The blood serum of each of these sows caused complete agglutination of a known culture of *B. abortus* Bang in a dilution of 1:100, with 75 per cent agglutination in a dilution of 1:250, and the complement was completely fixed with 0.02 cc. of the serum. Serum from a normal hog tested at the same time did not agglutinate the agglutinating fluid in any dilution, nor did it fix the complement.

The slipping of the pigs produced no after effects upon the sows that we could notice. They will be kept under observation for some time.

Taking into consideration all the results mentioned in this paper, we may conclude that the *Bacillus abortus* (Bang) is an etiological factor in infectious abortion of sows. Whether or not it is the only etiological factor, will have to be determined by further investigations. This is the second time, so far as we know, that the *Bacillus abortus* has been associated naturally with aborting animals of a species other than the cow. The first was discovered by Dr. Surface[3] when he found the disease epizootic among guinea pigs which were being reared in an inclosure in which inoculation experiments were being carried on with the Bang bacillus. Some of the litter from the cages containing the inoculated pigs had gotten into the pens of breeding pigs and caused the spread of the disease.

SUMMARY

1. Epizootic infectious abortion occurs occasionally among sows, though not so frequently as among cows and mares.

2. Previous to the time of this investigation, no etiological factor connected with the disease in the sow had been discovered.

3. In this investigation the *B. abortus* of Bang, the organism

[3] F. M. Surface, Bovine Infectious Abortion Epizootic Among Guinea Pigs, Journal of Infectious Diseases, 1912, 11, no. 3, p. 464.

causing the disease of infectious abortion in the cow, was isolated from the afterbirth of an aborting sow and from the contents of the umbilicus, heart, liver and stomach of two aborted fetuses.

4. The strain of *Bacillus abortus* isolated from the sow responded to all the biological and physiological tests of the strains isolated from the uterine exudate of aborting cows by this laboratory, with the exception that the original culture grew in the air after the first generation.

5. Pregnant sow no. 1, inoculated intravenously with 2 cc. of an agar slant culture of the bacillus secured from the aborting sow, washed off with 5 cc. of normal salt solution, aborted five fetuses seventeen days after the injection, and the organism was isolated from the afterbirth and internal organs of the fetuses. The bacillus in this instance grew directly from the tissues under strictly aerobic conditions. Pregnant sow no. 2, on being fed the organism derived from the aborting sow, aborted nineteen days afterwards. The sow ate the afterbirth and presumably some of the pigs. The organism was secured from the stomach contents of one of the fetuses obtained.

6. The blood serum of each of these sows, after aborting, completely agglutinated a strain of *Bacillus abortus* (Bang) derived from an aborting cow, in a dilution of 1:100. The complement was fixed in each case with 0.02 cc. of the serum. The serum of a normal hog did not agglutinate in any dilution, nor did it fix the complement.

THE RELATION OF PROTOZOA TO CERTAIN GROUPS OF SOIL BACTERIA[1]

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INTRODUCTION

The theory advanced by Russell and Hutchinson (1909; 1913) that protozoa are an important factor in limiting bacterial activity and consequent fertility in the soil has aroused no little interest. Their work has stimulated much investigation, some of the results of which seem to uphold their theory, while others do not substantiate it.

It was thought that results of interest might be obtained by further studies concerning the effect of the protozoa on certain biological processes of the soil—ammonification, nitrification and free nitrogen fixation.

For this study soil cultures of Miami silt loam soil from the Experiment Station Farm were used. The moisture content was maintained at as near one-half saturation as possible.

AMMONIFICATION

In this work 300 gram portions of soil were placed in each of 18 flasks. The flasks were of 500 cc. capacity, Erlenmeyer form, and rather narrow so that the soil was in approximately 2 inch layers. The proper amount of moisture was added, the flasks plugged, and sterilized at 15 pounds pressure for two hours. This was found sufficient to kill all the bacteria. Upon cooling, one half of the flasks were inoculated, each with 2 cc. of a suspen-

sion of normal soil known to contain protozoa. The remaining half were inoculated with the same amount of soil free from protozoa. The protozoa-free soil was obtained by sterilizing a portion of the Miami soil and inoculating it with as many kinds of bacteria as could be isolated by the plate method, using different kinds of media. The flasks were then incubated at room temperature (approximately 22° to 25°C) and the ammonia and nitrate content determined at the end of four, eight and twelve weeks. The ammonia was determined by distilling 100 grams of the soil with 10 grams of magnesium oxide and 250 cc. distilled water. The distillate was received into N/20 sulphuric acid and the excess acid remaining after the distillation was titrated with alkali of the same normality. The nitrate was determined by the phenolsulphonic acid method.

The results of this work are given in the following table:

TABLE 1

Ammonia and nitrate in soils with and without protozoa

TREATMENT	NITROGEN PER 100 GRAMS DRY SOIL					
	Ammonia			Nitrate		
	After 4 weeks	After 8 weeks	After 12 weeks	After 4 weeks	After 8 weeks	After 12 weeks
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
With protozoa.....	8.12	4.48	4.62	4.50	7.69	10.00
	7.70	4.69	4.90	4.50	7.69	10.00
	8.05	5.60	4.20	4.50	7.69	10.00
Without protozoa..	8.19	8.26	8.96	3.60	3.57	3.57
	8.10	8.19	8.96	3.60	3.57	3.57
	8.12	8.19	8.85	3.60	3.57	3.57

From the data of table 1 it will be noted that in the soil containing protozoa the ammonia decreased somewhat while in the soil free from protozoa it tended to increase to a slight extent. This may be explained by the fact that in the soil containing protozoa the nitrifying organisms were also present and functioning, whereas in the soil free from protozoa they were absent and thus the ammonia tended to accumulate. In the case of the nitrate formation, in the soil with protozoa the nitrate con-

tent naturally increased since the nitrifying organisms were present and the ammonia as it was formed was oxidized to nitrites and further to nitrates.   Where the soil contained no protozoa, the nitrate content remained practically unchanged because of the absence of the nitrifying bacteria.

In order to show the correlation between ammonia formation and nitrate formation the following table giving the total ammonia and nitrate nitrogen was compiled.

TABLE 2

Total ammonia and nitrate nitrogen in soils with and without protozoa

TREATMENT	AMMONIA AND NITRATE NITROGEN PER 100 GRAMS DRY SOIL		
	After 4 weeks	After 8 weeks	After 12 weeks
	mgm.	*mgm.*	*mgm.*
With protozoa......................	12.62	12.17	14.62
	12.20	12.38	14.90
	12.55	13.29	14.20
Without protozoa....................	11.79	11.83	12.53
	11.70	11.76	12.53
	11.72	11.76	12.42

From the summary data in table 2 it is very evident that the presence or absence of protozoa has very little effect on the sum total of ammonia and nitrate nitrogen.

On account of the absence of the nitrifying bacteria in the protozoa-free soil it is hardly fair to draw very definite conclusions from these results.   Therefore, other experiments were made, in which three sets of the same soil were used: (1) untreated, (2) heated to 90°C. for one hour and (3) heated to 90°C. for one hour and later reinoculated with 1 per cent of normal soil, thus introducing the nitrifying organisms and also the protozoa. These soils were incubated at room temperature and the ammonia and nitrate nitrogen determined every ten days for a period of thirty days.

The following results were obtained.

TABLE 3

Ammonia and nitrate in untreated, heated, and heated and reinoculated soils

TREATMENT	NITROGEN PER 100 GRAMS DRY SOIL					
	Ammonia			Nitrate		
	After 10 days	After 20 days	After 30 days	After 10 days	After 20 days	After 30 days
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Untreated............	2.10	1.82	3.50	4.63	5.55	9.80
Heated..............	8.96	9.80	11.75	2.50	2.51	3.03
Heated and reinocu- lated..............	8.96	10.08	6.44	2.96	6.66	8.33

As expected the ammonia content in the untreated soil remained quite constant while the nitrate increased. In the heated soil where the nitrifying bacteria and the protozoa were absent, the ammonia increased and the nitrate content remained practically the same. A part of the initial increase of ammonia in the heated and in the heated and reinoculated soils is undoubtedly due to the heating alone. The bacteria surviving this treatment cause some increase in ammonia as seen by the results obtained. But in the heated soil which was subsequently reinoculated with 1 per cent normal soil the ammonia decreased, due no doubt to its oxidation by the nitrifying bacteria, and the nitrate nitrogen increased. In the latter instance the protozoa were present, a factor which Russell and Hutchinson claim is detrimental to bacterial activity. The nitrifying bacteria were also present and active.

The following table compiled in the same manner as table 2

TABLE 4

Total ammonia and nitrate nitrogen in the untreated, heated, and heated and reinoculated soils

TREATMENT	AMMONIA AND NITRATE NITROGEN PER 100 GRAMS DRY SOIL		
	After 10 days	After 20 days	After 30 days
	mgm.	*mgm.*	*mgm.*
Untreated..............................	6.73	7.37	13.30
Heated................................	11.46	12.31	14.79
Heated and reinoculated................	11.92	16.74	14.77

shows the total ammonia and nitrate nitrogen present in the untreated, heated, and heated and reinoculated soils.

Here the introduction of the supposedly harmful factor, the protozoa, into the soil did not seem to have any depressing effect on the bacteria as far as their production of ammonia and subsequent oxidation of the same was concerned.

NITRIFICATION

Flask experiments were carried out somewhat similar to the ammonification tests, except that at the time of inoculation with soil containing protozoa and soil free from protozoa, the soil was also inoculated with cultures of *Nitrosomonas and Nitrobacter* free from ciliates and flagellates. The nitrifying organisms were obtained by inoculation and subsequent occasional transfer into media suitable to their growth and unfavorable to the growth of other organisms. After the inoculation of the sterilized soil as previously stated, ammonium sulphate was added in quantities equal to 20 mgm. of nitrogen as ammonia per 100 grams (dry weight) of the soil. The ammonia and nitrate content was determined at the end of fourteen and twenty-eight days.

The figures in tables 5 and 6 show the results of these determinations.

In the case of the soil with protozoa at fourteen days the average ammonia content was approximately 18.6 mgm. and at twenty-eight days, 16.0 mgm. Where the protozoa were ab-

TABLE 5

Rate of conversion of ammonia to nitrate in soil with and without protozoa

ANALYSES	TREATMENT	NITROGEN AS AMMONIA PER 100 GRAMS DRY SOIL	
		After 14 days	After 28 days
		mgm.	*mgm.*
1	With protozoa............................	20.58	15.82
2	With protozoa............................	19.02	15.96
3	With protozoa............................	16.10	16.24
1	Without protozoa.........................	21.14	19.32
2	Without protozoa.........................	23.24	19.18
3	Without protozoa.........................	20.16	19.32

TABLE 6

Rate of nitrate formation in soil with and without protozoa

ANALYSES	TREATMENT	NITROGEN AS NITRATE PER 100 GRAMS DRY SOIL	
		After 14 days	After 28 days
		mgm.	*mgm.*
1	With protozoa............................	9.90	13.88
2	With protozoa............................	11.90	13.88
3	With protozoa............................	11.76	13.51
1	Without protozoa........................	7.52	7.81
2	Without protozoa........................	6.57	8.06
3	Without protozoa........................	8.20	8.06

sent the relative decrease in the amount of ammonia was about the same, 21.5 mgm. at fourteen days to 19.3 mgm. at twenty-eight days. There seemed to be practically no difference in the rate of conversion of ammonia into nitrate in the two soils.

Where the rate of nitrate formation was determined the increase in nitrate formation seemed to be slightly in favor of the soil which contained the protozoa.

From the small amount of work done on the effect of protozoa on nitrification in soil, it seems that their presence is at least not detrimental to the process as determined by these experiments.

FREE NITROGEN FIXATION

Some very interesting results were obtained in this part of the work. Here both soil and liquid cultures were employed. The liquid medium gave the protozoa an environment better adapted to their growth and activity than did the soil cultures.

Soil cultures. Four hundred grams of soil were weighed out and spread in approximately one inch layers on six flat porcelain plates. These were then carefully covered with parchment paper and tied and then sterilized at 15 pounds for one hour. Upon cooling each culture was inoculated with a suspension of *Azotobacter* in sterile distilled water. Then one-half were inoculated with a suspension of soil containing protozoa and the remaining half with the same amount of protozoa-free soil. Af-

ter incubating at room temperature for one week, the soils in the plates were treated with 1 per cent of mannite. The mannite was thoroughly mixed with the soil by means of a sterile spatula. The plates were then incubated for three weeks at 25°C. The moisture content was kept as near one-half saturation as possible by the addition of sterile distilled water. At the expiration of the incubation period, the soils were placed in a 30°C. incubator until air dry. They were then ground in a mortar and thoroughly mixed and finally sieved. Duplicate total nitrogen analyses were made according to the modified Gunning method.

The results of the analyses are given in the following table.

TABLE 7

Rate of fixation of free nitrogen in soil with and without protozoa

ANALYSES	TREATMENT	TOTAL NITROGEN PER 100 GRAMS OF DRY SOIL	
		After 21 days	Average
		mgm.	*mgm.*
1	With protozoa............................	148.40	
		145.60	
2	With protozoa............................	150.50	147.45
		144.20	
3	With protozoa............................	149.10	
		144.90	
1	Without protozoa........................	147.00	
2	Without protozoa........................	144.20	145.02
		144.90	
3	Without protozoa........................	140.00	
		147.00	

From the data above it will be seen that there is a difference in total nitrogen in favor of the soil containing the protozoa. However, it is probably not marked enough to cause any difference in the fertility of the soil.

From these results it seems probable that the protozoa do not have any particularly harmful effect on the fixation of free nitrogen in the soil.

Liquid cultures. One hundred cubic centimeters of mannite

solution (Ashby's) were placed in each of ten liter Erlenmeyer flasks. To each of these flasks 10 grams of soil were added and the flasks and contents sterilized at 10 pounds for thirty minutes. After cooling each flask was inoculated with a suspension of *Azotobacter* in sterile distilled water. Finally, one half of the flasks were inoculated with 10 cc. of a suspension of 40 grams of normal soil in 400 cc. of sterile distilled water and the remaining half were similarly treated using protozoa-free soil. The flasks were incubated at 25°C. for three weeks. Previous to determining the total nitrogen the flasks were examined in order to ascertain whether or not protozoa were present. In those flasks inoculated with soil containing the protozoa they were present and in a very active state and in those inoculated with soil free from protozoa they were not found.

The results of the total nitrogen analyses are given below.

TABLE 8

Rate of fixation of free nitrogen in solution with and without protozoa

ANALYSES	TREATMENT	TOTAL NITROGEN PER 100 CC. OF SOLUTION	
		After 21 days	Average
		mgm.	*mgm.*
1	With protozoa..........................	31.78	
2	With protozoa..........................	32.20	
3	With protozoa..........................	31.36	31.74
4	With protozoa..........................	31.64	
1	Without protozoa.......................	33.60	
2	Without protozoa.......................	33.60	
3	Without protozoa.......................	34.61	33.79
4	Without protozoa.......................	— —	

The results of the total nitrogen determinations revealed a difference of 2.05 mgm. of nitrogen in favor of the cultures without the protozoa. Apparently the protozoa had a slight detrimental effect on nitrogen fixation in solution. The protozoa-free cultures contained 33.79 mgm. of nitrogen and the cultures with protozoa contained 31.74 mgm. of nitrogen.

Thus it appears that in liquid cultures where the protozoa are in an actively motile state they seem to exert a harmful

influence on the process of free nitrogen fixation. It is probable that the larger protozoa made use of the *Azotobacter* as food. In certain cases upon staining a small amount of the film from a liquid culture with Gram's iodine solution *Clostridium* cells could be very readily distinguished within the protozoan cell. The probable presence of *Azotobacter* cells within the protozoa cells was also observed but by no means as definitely as in the case of the *Clostridium*.

It may be concluded that the protozoa have a slight detrimental effect on free nitrogen fixation in solution because the individual determinations seem to check closely and to be quite outside the limit of experimental error considering the small amount of nitrogen in the cultures. In the case of the soil cultures such a slight difference is not so important because the total nitrogen content here is approximately five times that of the liquid cultures.

DISCUSSION

From the results of this study of the influence of the protozoa on ammonification, nitrification and free nitrogen fixation in soil it would seem that their effect can not be considered detrimental. This is in accord with the work of other investigators, even with that of Cunningham (1915) who claims that his results uphold the theory put forth by Russell and Hutchinson. In the work referred to he states that soil protozoa in solution exercise a decided limiting effect on the numbers of bacteria and that in an ammonifying solution they show their activity by causing a decrease in the amount of ammonia produced as compared with cultures free from protozoa. These results are in accord with those already presented in this paper, in regard to free nitrogen fixation in solution. The protozoa seemed to have a detrimental effect on this process but when experiments were carried out on free nitrogen fixation in soil, the protozoa did not appear to influence the amount of nitrogen fixed. Cunningham may well conclude that protozoa have a limiting effect on the number of bacteria in solution, for here an environment

is furnished for the protozoa which is never met with in soils under normal conditions.

The results of the work at the New Jersey Experiment Station are not in accord with Russell and Hutchinson's theory. Lipman et al. (1910) found that the protozoa do not play any important part in depressing the activity of the soil bacteria. This was shown by a series of experiments performed relative to a possible influence which the protozoa might have on the important soil process of ammonification.

Concordant results have been obtained by Sherman (1916) who worked with six species of protozoa, namely, the two ciliates *Colpoda cucullus* and *Balantiophorus elongatus*, which are not active in soil and four flagellates, which by test were shown to be active in soil. The ciliates had a very marked detrimental effect upon the number of bacteria in soil extract but had no effect upon them in soil. Three of the flagellates had no effect upon the number of bacteria either in soil extract or soil. The fourth flagellate had a very marked detrimental effect in soil extract but none in soil. These experiments were performed many times and always with the same results.

In an earlier work Sherman (1914) showed conclusively that some protozoa can increase in numbers in the soil under ordinary conditions but from the results of his later work it is probably doubtful if they have any appreciable effect on limiting the numbers of the soil bacteria.

Grieg-Smith (1912), drawing conclusions from his own work, thinks that the protozoa have but little effect on the bacteria of the soil. He tested the action of the soil phagocytes (the protozoa) in the same manner as Russell and Hutchinson did and from his experiments he concluded "that Russell's contention cannot be sustained; the protozoa have little or no action in limiting the number of soil bacteria."

Goodey (1911), working with the ciliates only, thinks that these protozoa do not exist in the soil in an active state, but that they are present in an encysted condition. He made a careful study of recently excysted *Colpoda cucullus* obtained from soil which had been added to a suitable medium but a few

hours before. He concluded that if these organisms had been in the soil in an active state they would have possessed food vacuoles, as these develop soon after the protozoan begins to ingest its food.

CONCLUSIONS

In conclusion it may be said that in the soil cultures the presence of protozoa under the conditions of the experiments did not have any noticeable effect, detrimental or otherwise, on the processes of ammonification, nitrification and free nitrogen fixation. In the case of the liquid cultures employed in the study of free nitrogen fixation the conditions were at an optimum for the development of the protozoa and under these circumstances they limited bacterial activity as evidenced by the harmful effect on the fixation of free nitrogen. Undoubtedly under these conditions the protozoa were active in destroying the *Azotobacter* cells. But in the soil cultures conditions were evidently not favorable for the activity of the protozoa as these organisms did not appear to exert any harmful influence on the three soil processes studied.

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A STUDY OF THE BOAS-OPPLER BACILLUS

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In 1895 Boas and Oppler (1895) published a paper in which they reported observations on a large bacillus occurring in the gastric juice of patients afflicted with carcinoma of the stomach. In the same year Schlesinger and Kaufmann (1895) found a similar bacillus in 19 cases out of 20 cases of gastric carcinoma examined. These findings were further confirmed by several investigators and the presence of large numbers of these bacilli in the stomach was taken to indicate carcinoma. Strauss (1895) reported finding similar organisms in normal gastric juice although in small numbers. Kuntze (1908) was the first to suggest that the Boas-Oppler bacillus was related to the lacto-bacilli. Rodella (1908) has also shown the similarity of the Boas-Oppler bacillus to the so-called acidophile or aciduric bacilli and the *B. bifidus* of Tissier. These suggestions were given further experimental support by the work of Heinemann and Hefferan (1908). The authors found in an extensive investigation of that group of bacteria, now commonly known as the *B. bulgaricus* group, that similar bacilli were present in two samples of normal gastric juice and in two cases of gastric carcinoma. These organisms are difficult to cultivate as they do not grow on ordinary laboratory media to an appreciable extent; but they grow well in milk or on media prepared from milk. The presence of glucose or some other carbohydrate favors growth. For a detailed description of the organism and its cultural characteristics the reader is referred to the publication of Heinemann and Hefferan (1908).

In 1914 Galt and Iles (1914) reported the finding of the same organism in three cases of gastric carcinoma. They thought that malignant cases of carcinoma could be distinguished from

benign ones by the presence of these bacilli. This conclusion was reached because in malignant cases the hydrochloric acid disappears, while lactic acid is frequently present.

Bacilli of the *B. bulgaricus* group are widely distributed in nature as shown by several authors. Heinemann and Hefferan have found them in the feces of man, horses and cows, in soil, in fodder for cattle (bran, silage, dry brewer's grains), in cornmeal, sauerkraut, olive juice, dill pickles, pepper mango, market milk and in human saliva. Hastings and Hammer (1909) reported the presence of these bacilli in milk, butter and cheese and recently Hunter and Bushnell (1916) ascribed the fermentation of silage to the activity of the *B. bulgaricus* group. That they are active in final stages of the ripening of Emmenthaler cheese has been shown by Eldredge and Rogers (1914) and they have been reported by Evans, Hastings and Hart (1914) in Cheddar cheese. Dotterer and Breed (1915) during a tour of inspection of cheese factories in New York State have found that the pasteurized whey undergoes a practically pure lactic acid fermentation due to *B. bulgaricus* and that in unpasteurized whey the organisms are present in enormous numbers. The authors state also that the pasteurization temperature applied to these cases (66°–71°C.) was not sufficient to destroy the organism, although it destroyed most other bacteria present.

Since lacto-bacilli have been found in saliva and feces under normal conditions, it would be surprising if they did not exist normally in the stomach. Their presence in the digestive tract is the natural result of their frequency in foods, especially milk and milk products. Furthermore, this group of bacilli is able to resist a relatively high degree of acidity and survive where other bacteria are largely destroyed. Consequently the hydrochloric acid of the gastric fluid is not destructive to them, although it undoubtedly restrains their growth.

That lacto-bacilli actually exist in normal gastric juice has been shown by Strauss and Heinemann and Hefferan as stated before. If the hydrochloric acid restrains multiplication, it seems logical to assume that reduction in the amount of hydrochloric acid or its absence will permit growth and this assump-

tion may explain the finding of lacto-bacilli in large numbers in cases of gastric carcinoma.

However, if it be true that the presence of lacto-bacilli in large numbers in the stomach is due to a reduced quantity of hydrochloric acid, it may be assumed that they ought to be easily found in any pathological condition which reduces the normal acidity of the gastric juice. This reasoning led us to carry on a study of the problem the results of which are presented in this paper. We were aided by the kindness of Mr. A. G. Bower who furnished samples of gastric juice from a variety of sources.

The method of isolation was the same as the one employed by Heinemann and Hefferan. About half a cubic centimeter of gastric fluid was inoculated into acetic acid broth and incubated at 44°C. After twenty-four hours incubation several loopfuls were transferred to litmus milk and this was also incubated at 44°C. After a further twenty-four hours, transfers were made from the litmus milk tubes to other similar tubes and this proceeding was repeated every twenty-four hours until the characteristic reaction in litmus milk was observed. The coagulum should be smooth and compact with the appearance of little or no whey; the major part of the milk should be decolorized and a surface layer of intense red appears. Stains with methylene blue were prepared to ascertain the presence of long bacilli in pure culture. In order to study colony formation, plates were prepared in whey agar. Maltose broth was inoculated with five strains (P, S, F, H and the strain from infant feces) to test their reaction on this carbohydrate and finally 500 cc. of sterilized milk were inoculated with the same five strains. The evolution of acid was determined by titrating 5 cc. of the undiluted milk with N/20 NaOH and the acidity was calculated as lactic acid. The flask inoculated with strain H (from carcinoma) became contaminated and the results had to be excluded. The optical rotation of the lactic acid produced was determined after preparing the zinc salts in the usual manner.

Since lacto-bacilli are present in saliva it was suggested by Heinemann and Hefferan that they might be identical with

Leptothrix buccalis, the organism thought to be the cause of caries of teeth. We examined the decayed portion of four teeth after having carefully cleaned the teeth with sterile NaCl solution to avoid the chance of lacto-bacilli being present in the dried saliva on the outside of the teeth. After cleansing the teeth the inside decayed part was scraped out with a sterile knife and placed in acetic acid broth. Otherwise the same technic was employed as with the other material.

Material for examination was obtained from the following sources:

SAMPLE NUMBER	CONDITION	KIND OF MATERIAL	STAIN FROM MATERIAL
1. P............	Gastric ulcer	Gastric juice	Many large granular bacilli
2. M............	Gastric ulcer	Gastric juice	Many large granular bacilli
3. F............	Gastritis .	Gastric juice	Many large granular bacilli
4. S............	Pernicious anemia	Gastric juice	Many large granular bacilli
5. H............	Carcinoma	Gastric juice	Many large granular bacilli
6. P............	No diagnosis	Gastric juice	Some large granular bacilli
7. J............	Normal	Gastric juice	Few large granular bacilli
8. Bottle-fed infant......		Feces	Many large granular bacilli
9. Breast-fed infant......		Feces	Few large granular bacilli
10. Tooth........	Abscess	Decayed part	No stain made
11. Tooth........	Pyorrhea	Decayed part	No stain made
12. Tooth........	Pyorrhea	Decayed part	No stain made
13. Tooth........	Ulceration	Decayed part	No stain made

From all these cases typical lacto-bacilli were isolated. After four to six transfers in litmus milk the typical appearance of the milk was observed. Stains were prepared from all cultures and the bacilli appeared as large, rather slender or fairly thick organisms with blue granules. They were Gram positive. The colonies were all of the compact type. Sandberg (1904) first called attention to the two kinds of colonies formed by the Boas-Oppler bacillus. One of these has woolly edges, the other is solid. Similar observations have been reported by several authors.

The progressive amount of acid formed is shown in the following table:

SAMPLE	ORIGINAL MILK	AFTER DAYS								
		1	2	3	4	6	9	13	17	20
I. P.........	0.14	0.22	0.66	1.12	1.34	1.56	1.56	1.56	1.56	1.57
4. S.........	0.14	0.20	0.43	0.93	1.21	1.38	1.41	1.42	1.48	1.49
3. F.........	0.14	0.25	0.90	1.27	1.29	1.32	1.32	1.33	1.35	1.36
8. Infant feces	0.14	0.25	0.39	0.57	0.71	0.95	0.98	1.03	1.05	1.05

The amount of acid formed by the different strains is remarkably uniform and the rate of acid formation nearly the same in the first three samples. The strain from infant's feces is somewhat slower in acid formation than those from pathological conditions.

In maltose broth the strains P, S and F produced no change in reaction, while the strain from infant feces produced 4 per cent normal acid or 0.36 per cent lactic acid in five days at 37°C. and the same amount in two days at 44°C.

The strain from carcinoma lost its power to coagulate milk after seven transfers. We are unable to give an explanation of this phenomenon, unless it was due to enfeebling of the organism.

We found typical lacto-bacilli in the decayed contents of the four teeth. In one of the teeth (sample 10) long bacilli forming filaments and showing granular staining were found in large numbers. A few streptococci were also present. In one of the pyorrhea cases (sample 11) the bacilli were somewhat shorter than in the previous case. There were some that stained solidly, while others showed distinct granular staining. In the second pyorrhea case (sample 12) the long form was prevalent and filament formation was common. The fourth tooth (sample 13) was decayed and an ulceration was present at the root. The lacto-bacilli present were rather slender, the granular staining form being prevalent. Streptococci were also numerous. In the cultures obtained from two of the teeth (samples 10 and 13)

branching forms resembling the letter Y were observed. In all cases of material obtained from teeth the typical milk reaction resulted after three transfers in litmus milk. On account of the granular staining we assume that the lacto-bacilli from diseased teeth belong to the low-acid type.

This investigation confirms the results of previous work indicating that the Boas-Oppler bacillus is a member of the group of lacto-bacilli. Its cultural characteristics are in harmony with the descriptions of the group given by various authors and it appears established that members of the *B. bulgaricus* group are present in saliva, diseased teeth, gastric juice and the intestinal contents. The source can undoubtedly be looked for in certain foods, especially milk and milk products.

The finding of lacto-bacilli in large numbers in carious teeth is perhaps not conclusive evidence that they are actually the cause of decay. Experimental evidence to prove this would of course be difficult to obtain. However, since that kind of decay of teeth is usually ascribed to the presence of relatively large quantities of acid and since normal saliva is of an alkaline reaction the assumption is not difficult to arrive at that lactobacilli may be the cause. This is further supported by the fact that Heinemann and Hefferan found large numbers of lacto-bacilli in a sample of saliva of acid reaction.

White and Avery (1909) have attempted to separate the group of lacto-bacilli into two types as follows: Type A stains homogeneously with Löffler's methylene blue and Neisser's stain, produces 2.7 to 3.7 per cent lactic acid in milk and the lactic acid formed is of the inactive variety. Type B, stained with Löffler's methylene blue or Neisser's stain shows intensely stained granules; the bacilli of type B produce 1.2 to 1.6 per cent lactic acid in milk and the lactic acid formed is always levo-rotatory. According to this classification the strains examined by us belong to type B.

Rahe (1914) has classified aciduric bacteria according to their ability to clot milk and produce acid from maltose. He distinguished three varieties, namely: Variety A which clots milk, but has no action on maltose; variety B which clots milk and

ferments maltose; and variety C which ferments maltose, but does not clot milk. Strains F, P and S of our series belong to variety A of Rahe and the strain from infant feces belongs to variety B.

The presence of lacto-bacilli throughout the digestive tract has some bearing on the hypothesis of Metchnikof that life can be prolonged by establishing bulgarian bacilli permanently in the digestive tube. Rahe (1915) studied the problem of implanting *B. bulgaricus* in the alimentary tract and came to the conclusion that it cannot be adapted to the human lower intestine and that in monkeys the *B. bulgaricus* is capable only of an apparently limited survival in the upper intestine. The author further claims that *B. bulgaricus* can be readily distinguished from intestinal aciduric bacteria by its lack of ability to ferment maltose.

With these facts before us it seems clear that thēre is no support for the theory that lactic acid *in statu nascendi* is of value in suppressing intestinal putrefaction. The chief difference between the lacto-bacilli normally present in the alimentary tract and typical *B. bulgaricus* is the smaller amount of lactic acid produced by the former. However, the actual quantity produced is about 1.5 per cent, an amount which is greater than can be assumed actually to exist in the digestive tube. If we consider further that earlier findings of *B. bulgaricus* in feces, even after ingestion of bulgarian milk, were not entirely trustworthy on account of imperfect technic and that no attempt was made to distinguish between the high-acid and low-acid types, it becomes evident that the existence of an appreciable quantity of lactic acid in the digestive tract as a result of bacterial action is at least questionable. There can be no reasonable doubt about the actual formation of lactic acid by bacteria in the digestive tract, but the acid is promptly decomposed and utilized by the system.

The question naturally presents itself whether the types A and B of White and Avery are permanent or the result of environmental conditions and changeable. Type A is represented chiefly by typical *B. bulgaricus*. It forms about twice as much

lactic acid of the inactive modification as type B which produces only levo-rotatory acid. Granular staining is frequently a characteristic of enfeebled forms of bacteria and it is possible that type B is an enfeebled strain of type A that has lost the power to form dextro-rotatory lactic acid. If equal amounts of both modifications of active lactic acids are formed they unite to form inactive acid and it might be possible to convert type B into type A if suitable conditions of growth were offered. Type B is the one that is found active in cheese ripening and this fact suggests that type B grows at lower temperatures than type A whose optimum temperature is about 45°C. It is usually stated that type A does not grow below 30°C. It is possible therefore that type B is a modification of type A accustomed to lower temperature. We hope to study this problem at some future period.

SUMMARY AND CONCLUSIONS

1. Boas-Oppler bacilli occur in normal gastric juice in moderate numbers and in gastric juice containing either no hydrochloric acid or materially less than the normal amount in large numbers.

2. The presence of Boas-Oppler bacilli in large numbers in gastric juice is an indication of reduction of the normal hydrochloric acid content, whether this is due to gastric ulcer, gastritis, pernicious anemia or gastric carcinoma and possibly other pathological conditions.

3. The Boas-Oppler bacillus belongs to the group of lactobacilli which occur frequently in foods, chiefly milk and milk products. It gains access to the saliva, the stomach and intestinal contents with food.

4. The Boas-Oppler bacillus is the lactic acid producing organism that occurs in saliva and in the contents of the digestive tube.

5. The Boas-Oppler bacillus is common in feces of bottle-fed infants, but relatively scarce in the feces of breast-fed infants.

6. Four strains of Boas-Oppler bacilli studied by us produced in milk from 1.05 to 1.57 per cent acid, consisting chiefly of

lactic acid.   No acid was produced in maltose broth by strains from pathological conditions.

7. The lactic acid produced by the strains of Boas-Oppler bacilli studied by us is of the levo-rotatory modification.

8. A strain isolated from the intestinal contents of a bottle-fed infant coagulated milk and produced 1.05 per cent (lactic) acid in twenty days.   In maltose broth 0.36 per cent (lactic) acid was produced by this strain.

9. Lacto-bacilli were found in material from decayed teeth from which desiccated mucus had been removed.   This fact suggests that *Streptothrix buccalis* is perhaps a lacto-bacillus of the low-acid forming type.

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A CONTRIBUTION TO THE BACTERIOLOGY OF SILAGE[1]

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The fermentation which ensilage undergoes during its curing process was looked upon a few decades ago as being entirely of microbic origin, and we find, in the older literature on the subject, frequent reference to the alcoholic, acetic acid and lactic acid ferments which were supposed to exist in the ensilage. The evidence upon which such statements were based was, as far as can be ascertained, merely the occurrence in silage of the products characteristic of the action of such organisms. At the present time, due chiefly to the work of Babcock and Russell (1900, 1901), opinion has swung in the opposite direction to such an extent that microörganisms are now generally considered of little if any significance in the normal fermentation of silage.

That most microbiologists in this country do not consider bacteria of significance in the formation of silage is indicated by a review of the recent textbooks on the subjects of general and agricultural bacteriology. Marshall (1911) and Jordan (1914) make no mention of silage, although other related fermented products are discussed. Russell and Hastings (1915) state that the fermentation is believed to be due to the respiration of the living plant cells. Lipman (1911) outlines the respiration theory of Babcock and Russell and states that silage may be made under conditions which exclude bacterial action.

He further states that the question as to whether bacteria have any beneficial function can not be answered at the present time. Conn (1901) gives the old ideas concerning the supposed rôles of microörganisms, and then reviews the work of Babcock and Russell (1900, 1901). His views on the subject may be well exemplified by the following quotation:

From all these facts it becomes clear that while this method of preparing food is due to a fermentation, it can not be attributed to the growth of microörganisms. It certainly involves other factors, and it is uncertain whether bacteria, or other microörganisms, have anything to do with the process as normally carried out.

Since the work of Babcock and Russell fifteen years ago, little has been published on the processes involved in the curing of ensilage. E. J. Russell's work (1908) was in substantial agreement with that of Babcock and Russell, though he thought it possible that bacteria play a minor part. Esten and Mason (1912) considered the process entirely bacteriological. Three chief fermentations were thought to take place: the lactic acid, alcoholic and acetic acid fermentation. The lactic acid fermentation was thought to be due to organisms similar to those concerned in the souring of milk. It was also believed by these workers that yeasts cause an alcoholic fermentation and that acetic acid bacteria then oxidize the alcohol so formed to acetic acid. Samarani (1913) concludes that the acetic acid fermentation in silage is due to the respiration of the plant cells, while the lactic acid fermentation is due to bacterial action. The organisms responsible for the latter process were identified by Samarani as a bacillus and a coccus which occurred in about equal proportions. The former he designated as the *B. acidilactici* of Hueppe, and the latter was considered identical with the common streptococcus of milk.

The rôle of acid producing bacteria of the Bulgarian type in such fermented products as sauerkraut, milk beverages, cheese, etc., is well known. That they should function in silage would appear probable, but until very recently no data were at hand which gave any evidence for such a belief. Although

suggestions that these organisms may be of importance in the ensiling of foods are found in the literature (Heinze, 1913), such suppositions have not been based upon established facts. The lactic acid bacteria mentioned by Esten (1910) as important in silage were inhibited by the presence of only 0.45 per cent of lactic acid when grown in corn juice. Gorini (1906) made a detailed study of the bacteria of silage and mentioned *Streptococcus lactis-acidi* and *B. lactis-acidi* as the most important of the acid-forming organisms. None of the organisms which he described had the property of forming a high degree of acidity. Löhnis (1907) in his classification of the lactic acid bacteria described a number of strains of the aciduric bacteria but made no mention of a group derived from silage. Stevenson (1911) studied the distribution of the high acid bacteria but did not report silage as one of the sources from which they were obtained. Heinemann and Hefferan (1909) noted silage as one of the substances from which they had isolated cultures of *B. bulgaricus*. The recent paper by Hunter and Bushnell (1916) however is the first report, so far as the writer is aware, of the constant occurrence and probable importance of organisms of the *B. bulgaricus* group in silage.

OBSERVATIONS

The notes recorded in this paper are those which have been made on ensilage, more or less incidentally, during the past year. It was first noted on April 26, 1915, that sterilized milk inoculated with silage developed a high acidity. After incubation for ten days at 37°C. the milk was found to contain an acidity of 2.3 per cent calculated as lactic acid. This observation indicated the presence of organisms belonging to the group of aciduric bacilli, and these bacteria were isolated from the milk culture by plating on ordinary lactose agar. Their occurrence has been repeatedly verified in samples of corn ensilage from four different silos taken at various stages during the feeding season.

That the high acid-producing organisms not only exist in

silage but that they occur in large numbers is shown by the
fact that sterile milk usually develops a high acidity when
inoculated with dilutions of silage representing only 1/1,000,000
of a gram of the moist material, or when inoculated with 1/1,000,-
000 of a cubic centimeter of the juice expressed therefrom.   The
occurrence of the high-acid organisms in such large numbers
has been observed in silage over nine months old.

The aciduric bacilli may also be readily isolated by the direct
plating of the silage on lactose agar on which this type can be
grown.   In fact these organisms constitute a majority of the
bacteria found in ensilage during the latter part of the curing
process.   Unfortunately, it was not possible to make a study
of the silage during the first two months when the most impor-
tant bacterial processes undoubtedly take place.   Plate counts
made on lactose agar of the juice expressed from silage between
three and six months of age have given counts ranging from a
few thousand to over 1,000,000 bacteria per cubic centimeter.
As is well known, most types of the aciduric bacilli do not grow
well on agar plates, and it would seem very probable that the
actual number of such organisms is considerably greater than
is indicated by the plate count.   That this supposition is cor-
rect is shown by the observation that these organisms may be
present in numbers approximating 1,000,000 per cubic center-
meter of juice, as revealed by the dilution method, when the
plate count shows only 15,000.

Counts made on silage juice by the direct microscopic method
of enumeration have shown the presence of from 1,500,000,000
to 4,800,000,000 bacterial cells per cubic centimeter, most of
which were slender rods.   Many of the organisms observed
under the microscope were, in all probability, dead, since autoly-
sis would undoubtedly be greatly retarded in such an acid me-
dium.   However, these observations indicate that immense
bacterial activity had taken place.

The morphological and cultural characteristics of the acid
producing bacilli which have been isolated agree with those
reported by Hunter and Bushnell (1916).   The rods were of
variable size, but the growth characters of the cultures thus

far collected are very similar in so far as the cultural observations have been made. The colonies on agar appear exactly like those of the *B. lactis-acidi* group and, in the presence of a fermentable carbohydrate, they are surrounded by the characteristic haze. The development of colonies is not so rapid as with organisms of the *B. lactis-acidi* type, but on prolonged incubation they usually develop to a greater size. The readiness with which this group of bacteria grows on ordinary laboratory media differentiates it quite sharply from the typical *B. bulgaricus* of milk.

Not only do these organisms cause a high acid fermentation in milk but they have a similar action in corn juice in which they grow very rapidly. In the table given below are the data

TABLE I

Acidity produced by silage organisms in milk and in corn juice

CULTURE NUMBER	PER CENT ACID AS LACTIC ACID	
	Milk	Corn Juice
1	1.36	1.36
2	2.25	1.67
3	1.38	1.21
4	1.53	1.67
5	2.34	1.35
6	1.36	1.53
7	1.51	1.69
8	2.28	1.25
9	1.39	1.44
10	1.44	1.69
11	2.25	1.24
12	1.34	1.55
13	1.39	1.64
14	1.53	1.51

obtained with fourteen cultures grown in milk and in corn juice. The corn juice used was obtained from green plants at about the tasselling stage. The juice was expressed from the stalks by pressure, heated for a few minutes in the autoclave, filtered through filter paper, tubed and sterilized. The cultures were incubated twelve days at 37°C.

The observations reported in this paper would appear to indicate that acid producing bacteria might play a part in the fermentation of silage. How much of a factor they are in ordinary silage can not be answered from the meager data which have been collected. From the evidence presented by various workers, there can hardly be any question but that cell respiration is of vital importance in the fermentation of normal silage, but that this may be supplemented greatly by the action of bacteria certainly appears reasonable. It would seem that microörganisms might be responsible for the fermentation which takes place in silage made from shocked corn. The ensiling of shocked corn and corn stover, a practice which has been in vogue to a limited extent in some localities for years, in which we would expect the plant cells to be inactive, must be largely dependent, it would seem, upon the action of bacteria.

A laboratory test on this point was made by ensiling some corn stover with double the amount, by weight, of water in a glass jar. The stover used had been shredded and baled and was about fifteen months old. After one month at laboratory temperature the jar was opened and the ensilage examined. The material had a clean acid odor quite typical of ordinary silage, but on comparison of the two it was found to lack a certain richness in aroma so characteristic of silage put up in the usual way The juice expressed from the stover silage had an acidity of 1.35 per cent, calculated as lactic acid, and a bacterial count on lactose agar of 1,700,000,000 organisms per cc. of which 600,000,000 were of the high acid producing type. A direct microscopic examination of the juice revealed a count of 11,000,000,000 bacteria per cc.

The subject of the fermentation in stover silage is under further investigation at this station.

The constant occurrence of a group of organisms in silage with characteristics which differentiate its members from other related groups is of interest from a biological as well as from the practical viewpoint. The question naturally arises as to how they gain entrance to the ensilage, or what is their habitat in nature. Observations made in this connection indi-

cate an intimate relationship between the corn plant and this group of aciduric bacilli. Juice expressed from corn cut a few weeks prior to the time it was ready for the silo underwent an acid fermentation, when kept in the laboratory, with the production of a high degree of acidity and an aroma resembling that of good silage. From this were then isolated cultures of organisms similar to those obtained from silage. Sterile milk inoculated with bits of corn stover, either from the leaves or pith, always develops large amounts of acid and from it the typical organisms may be obtained. On a sample of shredded corn stover nearly a year and a half old the acid forming bacilli were found in numbers approximating 1,000 per gram as determined by dilutions in sterile milk.

SUMMARY

The data presented in this paper suggest the probable importance of a group of acid-tolerant, acid-producing bacilli in the curing of corn silage.

The organism concerned, while closely related to the *B. bulgaricus* group of milk and the *B. acidophilus* group of the intestines, appears to differ somewhat from the typical members of these groups, notably by its comparatively abundant growth on ordinary laboratory media.

The microscopic examination of silage juice demonstrates the presence of immense numbers of bacterial cells (always over one billion per cubic centimeter), most of which are bacilli which resemble morphologically the high acid producing bacilli described above.

The aciduric bacilli of silage are constantly found in quite large numbers on corn fodder, so that silage made from corn is always amply seeded with these organisms.

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BOOK REVIEW

Laboratory Manual in General Microbiology. WARD GILTNER. Pp. 418. John Wiley and Sons. 1916.

It is refreshing to have a laboratory guide for the study of microorganisms with a new title. This Manual is the result of the teaching experience of the author and co-workers at the Michigan Agricultural College and aims to be a guide for the teaching of general microbiology.

I suspect that many teachers experience the same difficulty the reviewer has felt in adapting another's laboratory directions to his own classroom work. The present Manual will be found extremely helpful and suggestive in the hands of the teacher but its wide acceptance as a class book seems doubtful, partly because of its length, and partly because of its lack of emphasis on any particular field or group.

The Manual is divided into three parts: Part I, General Morphological and Cultural Methods with 53 exercises; Part II, Physiology of Microörganisms with 33 exercises; Part III, Applied Microbiology, including 1 exercise on Air, 4 exercises on Water and Sewage, 10 exercises on Soil, 10 exercises on the Dairy, 1 exercise on Plant Microbiology, and 14 exercises on Animal Diseases and Immunity. The Appendix is of great value, including directions for the preparation of special media, stains, various tables and other data.

The illustrations are admirable and an excellent selection of pertinent references is given at the end of each exercise.

The book may be highly commended to teachers, who will cull from it many valuable suggestions, and when sufficiently extensive courses in general microbiology are offered it may well be adopted in the classroom.

<div align="right">C. M. HILLIARD.</div>

ABSTRACTS OF AMERICAN BACTERIOLOGICAL LITERATURE

BACTERIOLOGY OF FOOD

Feeding Experiments with Bacterium Pullorum. The Toxicity of Infected Eggs. LEO F. RETTGER, T. G. HULL AND W. S. STURGES. Jour. Exp. Med., 1916, **23**, 475–489.

This investigation is divided into two phases: first, a study of the toxicity or disease-producing properties of *Bacterium pullorum* when administered orally; and, second, an investigation of the heat tolerance of this organism in infected eggs when prepared for the table by the usual processes of cooking. It was found that eggs which harbor *Bacterium pullorum* in the yolk in large numbers may produce abnormal conditions, when fed, not only in young chicks, but in adult fowls, young rabbits, guinea pigs and kittens. Many market eggs are infected with this organism and since such methods of cooking as soft boiling, coddling and frying on one side only do not render the yolks free from viable bacteria, the feeding of eggs thus prepared may be the cause of serious disturbances in persons who are particularly susceptible to such influences, and especially in infants. Inasmuch as the wide distribution of ovarian infection in the domestic fowl has come about only in the last few years, its possible danger to man is one of recent development.—B. W.

BACTERIOLOGY OF THE MOUTH

Observations upon the Endamoebae of the mouth; I. Endamoeba gingivalis (buccalis). C. F. CRAIG. Jour. Infect. Diseases, 1916, **18**, 220–237.

A general discussion of this protozoan, its historical significance, geographical distribution, morphological features, relation to *Endamoeba hystolytica*, and its pathogenic rôle. The author concludes that "it is more than doubtful that *Endamoeba gingivalis* is the cause of pyorrhea alveolaris and that it is yet too early to make positive statements regarding the relation of this parasite to disease."—P. B. H.

Pyorrhea Alveolaris and Some Modern Discoveries Regarding Its Causes and Treatment. F. E. STEWART. New York State Jour. Med., 1916, **16**, 71–79.

In a comprehensive discussion of pyorrhea alveolaris or Rigg's disease, the author calls attention to the prevalence of the condition, and its importance as an etiological factor in many systemic diseases. Microörganisms, either of virulent types or those ordinarily saprophytic in the mouth, may invade the living tissues and cause the infection.

Associated with pyorrhea alveolaris, various types of bacteria have been recognized for some years, and recently certain protozoa. A review of the more important contributions on the bacteriology and protozoology of these cases is given. Under treatment the use of ipecac and emetine as an amebicide against *Entameba buccalis* is discussed, likewise the use of ammonium bifluoride, as recommended by Head, as a bactericide. The use of bacterial vaccines (bacterins) is indicated, especially when the condition does not respond to local treatment, or when it becomes systemic. The technique of collecting the material for, and the preparation of, bacterial vaccines is outlined. In considering the use of autogenous and stock vaccines the author holds that in certain cases a polyvalent stock vaccine is sufficient. A discussion of the theoretical principles of immunity in relation to vaccine therapy is presented.—L. W. F.

BACTERIOLOGY OF SOILS

Variation in Nodule Formation. L. T. LEONARD. Jour. Amer. Soc. Agronomy, 1916, **8**, 116.

Varying degrees of susceptibility between varieties of the soy beans to inoculation regarding the production of nodules were investigated. Nineteen varieties of soy beans were taken and inoculated with 1 cc. of a broth culture of the soy bean organism. From the results obtained, the author draws the conclusion that a soy bean culture isolated from a single strain of soy beans will be successful in inoculating any of the varieties studied.—A. I.

Nitro-cultures and their Commercial Application. F. C. HARRISON. Trans. Roy. Socy. Can., 1915, Ser. III, 9, Sec. IV, 219–223.

The writer refers to the paper presented to the Royal Society in 1906 by himself and Barlow on "The Nodule Organism of Leguminosae—its Isolation, Cultivation, Identification and Commercial Application." He reviews the commercial application of the methods then described, and notes that several firms in the United States have manufactured and distributed nitro-cultures according to these methods. The Ontario Agricultural College and the Macdonald College, Quebec, have sent out some 32,000 cultures with very favorable results, particularly with Alfalfa and Red Clover. Further experiments with media are reported, and the following revised medium which has been used successfully for the past two years is recommended.

Three solutions are prepared:

A. 75 grams of agar are dissolved in 3000 cc. of water, by placing in the autoclave at 10–15 pounds pressure.

B. 25 grams of hard wood ashes are boiled in 1000 cc. of water and filtered.

C. 0.5 gram of acid potassium phosphate, 0.5 gram of magnesium sulphate, 0.5 gram sodium chloride, 0.25 gram calcium sulphate, 6.25

grams of calcium carbonate are dissolved in the order named in 1000 cc. of hot water (about 80°C.).

The three solutions, A, B and C are mixed and 87.5 grams saccharose and 12.5 grams of mannite are added. The resulting 5 litres of medium are filled into ounce and a half wide mouth Blake bottles, plugged with cotton. These bottles when filled are sterilised in the autoclave at a pressure not exceeding 10 pounds. On removal, the bottles are sloped, and inoculated by means of a pipette. About 2 cc. of a suspension of the desired organism is run into each bottle. The bottles are kept in a sloped position, and incubated at 25°C. for about a week, when they are ready for distribution.—W. S.

BACTERIOLOGY OF WATER AND SEWAGE

Experiences in the Application of the Activated Sludge Process to Chicago Stockyard Sewage. ARTHUR LEDERER. Eng. Contrg., **45**, 388.

The temperature of the liquid affects the action in winter. The turbidity removal is an index of degree of treatment, and with the relative stability test aids in control.—L. P.

Winter Experience with the Activated Sludge Process at Milwaukee. W. R. COPELAND. Eng. Contrg., **45**, 386–387.

In winter with a sewage averaging 10.5°C. (minimum 4.5°C.) the oxidation was retarded. Two and one-fourth cubic feet air per gallon removed 90 per cent of bacteria and reduced the suspended matter to 15 parts per million. The sludge contained 5 per cent N as NH_3 and could be readily pressed. Analytical details are given.—L. P.

Grease and Fertilizer Base from Boston Sewage. R. S. WESTON. Jour. Am. Pub. Health Assn., 1916; **6**, 334–343; Eng. News, 1916, **75**, 913.

The use of SO_2 or H_2SO_4 on Boston sewage disinfects the liquid and precipitates most of the grease with the greater part of the suspended matter. Experiments showed a recovery of 1738 pounds dry sludge per million gallons containing 21.7 per cent grease. The estimates show an apparent profit of $6 per million gallons. Further investigation is recommended.—L. P.

The Sterilization of Chicago's Water Supply. Eng. Contrg., 1916, **45**, 18.

After 5 years trial, liquid Cl plants are to be installed on all intake lines. With less danger of tastes and odors, liquid Cl is cheaper, more flexible and capable of more precise control and reliability than other disinfectants.—L. P.

Maryland Experiences in the Disinfection of Water Supplies. R. B. MORSE. Eng. Contrg., **45**, 396–397.

From 0.2 to 3.0 parts per million of available Cl have been used in hypochlorite plants and 0.25 to 0.67 in liquid Cl plants. Fewer

complaints have been noted with liquid Cl. With intelligent operation the plants afford a cheap method of treatment for supplies not requiring filtration, and cause a marked decrease in typhoid.—L. P.

Copper Sulfate Treatment of St. Paul, Minnesota Water Supplies. N. L. HUFF AND G. O. HOUSE. J. Am. W. W. Assoc., 1916, **3**, 581–621.

One part per 12,000,000 parts of water was found to be adequate to eliminate *Spirogyra, Cyclotella* and most Cyanophyceae. One part per 10,000,000 remained effective for five weeks after which time treatment was repeated. Charts accompany the paper.—F. W. T.

The Water Supplies of Interstate Common Carriers on the Great Lakes. H. P. LETTON. J. Am. W. W. Assoc., 1916, **3**, 364–384.

Letton discusses mechanical features of the subject together with various methods for treatment of such water. Bacteriological analyses were made according to the method promulgated by the Secretary of the Treasury (Public Health Reports, November 6, 1914, p. 2960). From these bacterial examinations Letton considers it an impossibility to obtain a drinking water for boats directly from the lakes that will at all times conform to the Treasury Department standard.—F. W. T.

The Use of Ammonia in the Chlorination of Water. JOSEPH RACE. Can Engr., 1916, **30**, 345–346.

Ammonium hypochlorite proved to have a very rapid action, 20 to 30 times as rapid as $CaOCl_2$. The ammonium salt is not absorbed readily, preventing after-growths. The use of ammonia (aqua 16°B.) with $CaOCl_2$ may be economical when the $CaOCl_2$ costs over \$2.08 per 100 pounds. Difficulties in application require quick mixing with large dilution after ammonia is added to bleach solution to avoid loss.

L. P.

Some Aspects of Chlorination. JOSEPH RACE. J. Am. W. W. Assoc., 1916, **3**, 439–449.

Among other subjects Race discusses the questions of aftergrowths and types of *B. coli* which survive chlorination. Much difference of opinion exists with regard to the question of aftergrowths. The aftergrowths, under usual working conditions, vary according to the dosage of chlorine employed. A small amount of chlorine exerts a selective action. With large amounts a flora of spore formers survives which is unlike the original flora of the water. A study to determine whether the *B. coli* found after chlorine treatment were more resistant, indicated that there was little difference in the resistance of various types to chlorine.—F. W. T.

A Study of Some Organisms Which Produce Black Fields in Aesculin— Bile-Salt-Media. F. C. HARRISON AND J. VANDERLECK. With 4 plates. Trans. Roy. Socy. Can., 1915, Ser. III, **9**, Sec. IV, 207–217.

Six hundred pure cultures were isolated from aesculin bile-salt-agar, and lactose litmus agar plates made during analyses of 1000 milk sam-

ples from dealers or farmers in the Province of Quebec. These cultures were used to test the reliability of aesculin bile-salt-agar for the identification of the colon-aerogenes group. Of the 600 colonies many were selected because they seemed to be slightly atypical. There were only 10 exceptions; a percentage of 1.5 of all colonies tested; and a percentage of 0.03 of the colonies on the plates from which the 600 were isolated. Seventeen of the sub-cultures were subjected to the usual classification tests, and the results together with information as to the source of the milk are given in tabular form. Drawings showing the appearance on aesculin bile-salt-agar plates of surface and deep colonies respectively are included on plates I–IV.—W. S.

DISINFECTION

Further Investigation into the Precipitation of the Typhoid Bacillus by Means of Definite Hydrogen-ion Concentration. C. F. KEMPER. Jour. Infect. Diseases, 1916, **18**, 209–215.

The aim of the present work was to ascertain whether there exists a specific hydrogen-ion concentration for the precipitation of all strains of *B. typhi*. None was found; the effective range of hydrogen-ion concentration was wide, most strains being precipitated in the presence of a 3.6 by 10^4 hydrogen-ion concentration derived from the dissociation of acetic acid in the presence of sodium acetate.—P. B. H.

Effect of Pasteurization on Mold Spores. CHARLES THOM. J. Agr. Res., 1916, **6**, 153–166.

The object of this investigation was to see whether mold spores could resist the heat applied in pasteurization. Several species of Aspergillus and Mucor, and a large number of species of Penicillium together with a few other kinds of molds were tested. It was found that nearly all the mold spores were killed either by the holder process (30 minutes at 63°C.) or by the flash process (30 seconds at 74° or 80°C). The flash process at 74°C. proved the most efficient, only occasional spores surviving.

The effect of dry heat was also investigated. Dried preparations of the spores were submitted to various degrees of temperature. The resistance to dry heat was found to be much greater than to moist heat, considerable numbers of the spores surviving unless temperatures of 120°C. (for 30 seconds) were used.—H. J. C.

The Physical Chemistry of Disinfection. J. F. NORTON AND PAUL H. HSU. Jour. Infect. Diseases, 1916, **18**, 180–194.

This contribution attempts to apply physico-chemical methods to the problem of the mechanism of disinfection; and in the first place to demonstrate the rôles which undissociated acids, hydrogen ions and anions play in the process. The first test was made with formic acid, using *B. typhi* as the test organism. The results seemed to justify the following conclusions: that acids act as disinfectants through the agency of the hydrogen ions, and that the disinfecting power is proportional

to the H ion concentration. The addition of a salt possessing a common anion diminishes the power through decrease in the H ion concentration and increase in the concentration of the undissociated acid molecules. Salts which do not appreciably affect the dissociation of an acid greatly increase the disinfecting properties. In acid disinfection acid anions are positive catalyzers and undissociated acid molecules are negative catalyzers.—P. B. H.

The Bactericidal Properties of the Quarternary Salts of Hexamethylenetetramine. I. The Problem of the Chemotherapy of Experimental Bacterial Infections. W. A. JACOBS. Jour. Exp. Med., 1916, **23**, 563–568.

Introductory to subsequent papers. A discussion of the factors to be considered in formulating a program for systematic studies in chemotherapy.

Ibidem. II. The Relation between Constitution and Bactericidal Action in the Substituted Benzylhexamethylenetetraminium Salts. W. A. JACOBS, M. HEIDELBERGER AND H. L. AMOSS. Jour. Exp. Med., 1916, **23**, 569–576.

By the addition of substituted benzyl halides to hexamethylenetetramine, a series of quarternary salts of this base was obtained. These salts represent a new group of organic bactericides. The results obtained in the tests with these substances upon *Bacillus typhi* have demonstrated the existence of direct relationships between chemical constitution and bactericidal action within the series. The bactericidal character is directly attributable to the presence of the hexamethylenetetramine nucleus. The degree of the bactericidal action, however, is determined by the position, character, and number of the groups substituted in the benzene nucleus. By the introduction of the methyl, chlorine, bromine, iodine, cyano, and nitro groups into the benzene nucleus of the parent benzyl hexamethylenetetraminium salt, the bactericidal power of this compound was notably enhanced. The substitution of these groups in the *ortho* position almost invariably resulted in substances which were more active than their *meta* or *para* isomers. The introduction of the methoxy group was without marked effect. Several substances in which two hexamethylenetetraminium sidechains occurred were found to be the most active of the substances of this series when tested against *Bacillus typhi*. Comparative tests with other bacterial types demonstrated that these compounds possessed a marked degree of specificity for *Bacillus typhi*.

Ibidem. III. The Relation between Constitution and Bactericidal Action in the Quarternary Salts Obtained from Halogenacetyl Compounds. W. A. JACOBS, M. HEIDELBERGER AND C. G. BULL. Jour. Exp. Med., 1916, **23**, 577–599.

The extension of the study of the quarternary salts of hexamethylenetetramine to those obtained by the addition of this base to the most

varied types of substances containing aliphatically bound halogen has demonstrated that the introduction of the hexamethylenetetramine nucleus in this manner results in the production of bactericidal substances or enhances the bactericidal action if already present.

In particular it was found possible by the use of the halogenacetyl group, XCH_2CO, as a connecting link, to furnish primary and secondary aliphatic and aromatic amines, alcohols, and hydrocarbons of the most varied character with the hexamethylenetetramine molecule and to study the relation between chemical constitution and bactericidal action in the series of substances so prepared. Because of the variety of chemical types studied, the results are too involved for a detailed summary here. Many of the substances were found to be very powerful bactericides, and in a number of instances derivatives of purely aliphatic nature were found to possess an unusual bactericidal power. *Bacillus typhi*, streptococci, meningococci, and gonococci were the microörganisms used for the tests, and striking instances of partial specificity were observed. This specificity was found to favor not one species alone, but instances were found in which each of the types of bacilli was shown to be especially susceptible to one or another of the particular types of compound employed. The source of this partial specificity is to be sought not in the hexamethylenetetramine nucleus itself but in the molecule to which it is attached. The action of some of the substances was tested in the presence of serum or protein and was found to be not at all or only slightly i ihibited. In other cases marked inhibition occurred. The factors controlling the serum— or protein—compatibility of these substances are likewise to be sought in that portion of the molecule other than the hexamethylenetetramine.

B. W.

IMMUNOLOGY

Anti-Typhoid Inoculation. Eng. News, 1916, **75**, 530.

The Canadian Pacific Railway has used inoculation with success, reducing cases in two years to 3, as compared with 290 for two years among non-inoculated.—L. P.

Development of Immune Reactions in Serum Disease. W. T. LONGCOPE AND F. M. RACKEMANN. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 101–102.

Eleven patients, who had received horse serum for therapeutic purposes, were studied by two methods. Skin sensitiveness to horse serum was tested by intracutaneous injections of 0.02 cc. of horse serum, both undiluted and diluted ten or one hundred times with salt solution. Second, anaphylactic antibody was tested for by injecting the serum of the patient into guinea pigs and testing these for passive sensitization. The results show that anaphylactic antibodies for horse serum appear in the blood serum in maximum concentration towards the close of serum sickness and suggest that their presence determines recovery from this disease.—W. J. M.

Immunization with Sensitized Bacteria. HOMER F. SWIFT AND RALPH
   A. KINSELLA. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 103.

Two strains of green-forming streptococci were used. Rabbits were
injected with sensitized vaccines and compared with other rabbits in-
jected with unsensitized vaccines. In the latter group of animals there
was strong formation of antibodies in from twelve to sixteen days. The
animals injected with sensitized vaccines, on the other hand, showed
only weak agglutinins or complement-fixing bodies and the serum was
without protective value for mice.—W. J. M.

Complement Fixation in Acne Vulgaris. ALBERT STRICKLER, JOHN
   A. KOLMER AND JAY F. SCHAMBERG. Jour. Cutan. Dis., 1916, **34**,
   166–178.

The authors call attention to the indefinite bacteriology of acne
vulgaris and the probable etiological rôle of colon bacilli in this dis-
ease. They attempted a study of the relationship to the disease of
the bacillus of acne, the cocci from acne lesions and of *B. communis*
and *B. communior* from the feces of persons suffering with this disease,
by means of complement fixation tests using the same antigens with
the sera of normal persons and persons suffering with non-acneiform
diseases and controlling the results in acne by testing the serum of their
patients with polyvalent antigens prepared with cocci from furuncles
and with colon bacilli from the feces of healthy persons.

Of 57 cases of acne vulgaris, 84.2 per cent reacted positively with an
antigen of *B. acne*; 64 per cent reacted positively with the antigen of
staphylococci from acne lesions and practically the same results were
observed with the control antigen of staphylococci; 63.1 per cent
reacted positively with the antigen of *B. coli* from the feces of acne pa-
tients and 32 per cent reacted positively with the antigen of *B. coli*
from the feces of normal and healthy persons.

The sera of normal and syphilitic persons reacted uniformly negatively
with all antigens; the sera of persons suffering with various skin dis-
eases likewise reacted negatively in the majority of instances except
those with acne rosacea and seborrholic dermatitis.

From these studies the authors conclude that *B. acne* may be an
etiological factor in skin diseases other than acne vulgaris; that the
cocci found in these lesions possess no peculiar serological characteris-
tics such as would differentiate them from other staphylococci found
in furunculosis and that *B. coli* appears to exert an etiological influence
in some diseases of the skin and particularly acne vulgaris.—J. A. K.

*The Variations in Reaction of the Blood of Different Species as Indicated
   by Hemolysis of the Red Blood Cells when Treated with Acids or Alkalies.*
   J. G. CUMMING. Jour. Infect. Diseases, 1916, **18**, 151–179.

It had been shown in earlier work that the sign of the Wassermann
reaction might be reversed by the influence of salts, acids and alkalies
on the hemolytic system. The aim of the present work was to estab-
lish a chemical standardization for definite hemolytic time indices of

different animal species, as a preliminary to determining the percentage of ammonia, sodium hydroxide, hydrochloric acid and other inorganic and organic compounds and salts, necessary to cause complete hemolysis under certain arbitrary conditions. For a fifteen minute hemolytic system there was a marked difference in the requirements for NH_3, NaOH, and HCl, for some species but not for others. Various hemolytic time indices for the substances mentioned were worked out, and by this means it was found possible to identify blood cell suspensions of different species with considerable accuracy. The following conclusions were drawn: Alkaline hemolysis may be considered due to the hydroxyl group, while acid hemolysis is due to the H-ion. "The hemolysis of the red blood cell may be used an as indicator to ascertain the degree of acidity or alkalinity of certain solutions." "Alkaline hemolysis can be influenced by acids and acid hemolysis by alkalies. Both can be influenced by the neutral salt content of the suspension." It was shown that there was a distinct variation between the normal and the pathologic blood of the same species, since the time indices of the latter specimens were increased or decreased. It is suggested that this may be due to increased alkalinity or decreased acidity, or to variation in the natural salt content.

P. B. H.

INDUSTRIAL BACTERIOLOGY

The Removal of the Natural Impurities of Cotton Cloth by the Action of Bacteria. B. S. LEVENE, Journ. Ind. and Eng. Chem., 1916, 8, 298.

Levene investigated the possibility of removing the nitrogenous and fatty impurities of cotton fiber by means of bacteria in place of the vigorous chemical treatment now employed. After preliminary experimentation the following organisms were found most suitable: *B. amylolyticus, B. fimi, B. bibulus, B. carotovorus, B. subtilis.*

These bacteria are capable of hydrolyzing starch, and decomposing cellulose or pectin or both.

Coarse cotton cloth was sterilized in nutrient broth inoculated with the above named bacteria and incubated at 37.5°C. Tests were made after one, two, and three months respectively. Slight changes were observed after one month, more marked effects after two months, and decided alterations after three months. Cloth washed and bleached was perfectly white and was not yellowed by steaming. Chemical tests showed complete removal of nitrogenous impurities, about 80 to 90 per cent of the ether soluble impurities, and from 2.5 to 40 per cent of the alcohol soluble substances. The effect on the last mentioned substances varied with the different types, *B. caratovorus* being the most effective. Two forms—*B. bibulus* and *B. fimi*—caused weakening of cloth; the others apparently had no such effect.

By using different combinations of organisms and different media the incubation period could be reduced to 24 to 72 hours.—I. J. K.

MEDICAL BACTERIOLOGY

The Pure Cultivation of Spirochaeta Icterohaemorrhagiae (Inada). T. ITO AND H. MATSUZAKI. Jour. Exp. Med., 1916, **23**, 557–562.

This organism, claimed to be the causative agent of Weil's disease, was obtained by the authors in a solid, a semi-solid, and a fluid medium, enriched with blood. The spirochaete thus isolated remains pathogenic for guinea pigs for many generations. The characteristics of three strains are described.—B. W.

A Milk-Borne Paratyphoid Outbreak in Ames, Iowa. MAX LEVINE AND FREDERICK EBERSON. Jour. Infect. Diseases, 1916, **18**, 143–150.

The authors explain a slight epidemic on the grounds of a paratyphoid B. infection originating either from a carrier or from a convalescent. The paratyphoid cultures isolated, it is stated, produced gas in glucose broth.—P. B. H.

A Leptothrix Associated with Chronic Hemorrhagic Nephritis. G. R. DICK, G. F. DICK, AND B. RAPPAPORT. Jour. Infect. Diseases, 1916, **18**, 216–219.

The authors report a case of chronic nephritis characterized by recurring attacks of hematuria probably caused by a Leptothrix which was present in the urine. The causal relation was suggested by the constant occurrence of the organism, its virulence for animals and the improvement in the case which took place under vaccine treatment.
P. B. H.

Technique of Cultivating Human Tissues in Vitro. R. A. LAMBERT. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 100–101.

Chick plasma with the addition of an equal quantity of human serum furnishes a satisfactory medium, in which the fibrin network resists digestion. Human tissue may be preserved in viable condition for five to ten days, by immersion in salt solution in a cool place. The destruction of bacteria in infected tissues by means of chemical disinfectants is being investigated.—W. J. M.

The Cytology of the Exudate in the Early Stages of Experimental Pneumonia. FRANK A. EVANS. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 99–100.

Pneumonia was induced in rabbits by intrabronchial injection of pneumococci and streptococci and by the injection of egg yolk. The exudate in each instance contained many polynuclear cells but more often the predominant cells were mononuclear.—W. J. M.

Pneumotyphoid, with Report of a Case. F. BILLINGS. Am. Jour. Med. Sc., 1916, **151**, 36–39.

Billings reports a case which "seems to present an example of typhoid fever presenting its primary manifestations in the lung in the form of

a frank, outspoken croupous pneumonia." Cultures from the blood, and from the sputum yielded *B. typhi*, but the organism was not found in the stools or the urine.—L. W. F.

The Mode of Infection in Pulmonary Distomiasis. KOAN NAKAGAWA. Jour. Infect. Diseases, 1916, **18**, 131–141, Pls. II–IV.

The author reports on the distribution, origin and means of infection in the case of the distomiasis observed in Formosa. It is shown that at least two species of crabs found in Formosa and Japan proper contain in the liver or gills large numbers of encysted larvae, and that the number of infested crabs in any district is roughly proportional to the number of cases of distoma infection. Dogs were successfully infected as a result of eating liver or lungs of infested crabs. In the final host it was shown that the encysted larvae, after entering the intestines, reach the abdominal cavity by perforating the intestinal wall near the jejunum. They then penetrate the diaphragm and pleura and finally pierce the lung parenchyma. Here they develop and lay eggs which are discharged with other degenerative tissue products through the trachea.—P. B. H.

The Reactions between Bacteria and Animal Tissues under Conditions of Artificial Cultivation. II. Bactericidal Action in Tissue Cultures. H. F. SMYTH. Jour. Exp. Med., 1916, **3**, 265–274.

In a previous paper the author described an original method for studying the action of tissue cultures *in vitro* when inoculated with living pathogenic bacteria. In the present experiments chick embryo tissues were grown in a mixture of equal parts of plasma and Ringer solution. It was found that chicken plasma exerts a marked bactericidal action on *B. typhi* and on *B. diphtheriae* but is less marked with *B. dysenteriae*, and slight, if present at all, with *B. coli*. Chick tissues, particularly splenic tissue, counteract this action. The migrating white cells from splenic cultures have a distinctly bactericidal influence on all organisms tested except *B. coli*.

Ibidem. III. The Action of Bacterial Vaccines on Tissue Cultures in Vitro. H. F. SMYTH. Jour. Exp. Med., 1916, **3**, 275–291.

In plasma tissue cultures *in vitro* with tissue containing lymphatic elements the changes characteristic of early tubercle formation may be seen when such cultures contain masses of tubercle bacilli.—B. W.

Experiments with Poliomyelitis in the Rabbit. M. J. ROSENAU AND L. C. HAVENS. Jour. Exp. Med., 1916, **23**, 461–474.

The poliomyelitic virus obtained from an experimental monkey was passed through eight generations in rabbits with no apparent change in virulence. It is filterable and is virulent only for young rabbits. Even in these only about 40 per cent succumb. Inoculations were made intracranially, intravenously, into the sheath of the sciatic nerve and by placing the virus upon the uninjured nasal mucosa.

The incubation period varied from two to forty-one days with an average of twelve days. The lesions produced while definite and consistent lack the distinctive features of the pathologic picture of poliomyelitis in man and the monkey. The symptoms differ in individual rabbits and show variations from those seen in the monkey and in man.—B. W.

The Protection of Pathogenic Microörganisms by Living Tissue Cells. P. ROUS AND F. S. JONES. Jour. Exp. Med., 1916, 23, 601–612.

One series of experiments was carried out to determine whether phagocytes protect ingested bacteria against the bactericidal action of serum and of potassium cyanide. While the conditions of the tests are scarcely comparable with conditions in the body, yet they point to a protecting action on the part of the phagocyte. When erythrocytes and a hemolytic system were substituted for bacteria and bactericidal substances the results were sharp and conclusive. Suspensions of dog leukocytes were incubated with rat erythrocytes and dog serum was added for its opsonic action. After an hour's incubation anti-rat erythrocyte serum was added. It was then found that while all extra cellular rat erythrocytes were dissolved, the phagocyted red cells remained unaffected. From their experiments the authors conclude that living phagocytes are able to protect ingested organisms from the action of destructive substances in the surrounding fluid, and even from a strong homologous antiserum, and that this protection by phagocytes is largely, if not entirely, conditioned on their being alive. These findings should be taken into consideration in the study of diseases caused by infectious agents capable of living within tissue cells.—B. W.

Chemical versus Serum Treatment of Epidemic Meningitis. SIMON FLEXNER AND H. L. AMOSS. Jour. Exp. Med., 1916, 23, 683–701.

The authors studied the therapeutic effect of lysol and protargol in experimental meningococcus infections. Neither substance proved to have any curative action on the experimental infection in guinea pigs and protargol failed to influence favorably the infection following sub-arachnoid inoculation in monkeys. It was found that both lysol and protargol exert antileukotactic and antiphagocytic effects, and are also potent protoplasmic poisons, and the leukocytes with which they come in contact are injured and made to degenerate. The mixture of antiserum with lysol and with portargol reduces to a certain extent the antileukotactic and antiphagocytic effect of the chemicals; but this action is insufficient wholly to set aside the injurious effects which they produce. Any theoretical advantages they may possess are more than offset by the harmful effects which they cause, hence specific antiserum seems to provide the logical therapeutic agent with which to combat epidemic meningitis, since it is itself innocuous and promotes those processes essential to recovery from the disease.

B. W.

Further Experimentation in Animals with a Monilia Commonly Found in Sprue. B. K. Ashford. Am. Jour. Med. Sc., 1916, **151**, 520–528.

This report is a continuation of the author's studies in experimental moniliasis in animals by means of a new monilia found in sprue. He feels that sufficient evidence has been gained to justify Bahr's opinion that sprue is due to a monilia, but not *Monilia albicans*, since he has found in Porto Rico a distinct, undescribed species in nearly one hundred cases of true sprue, and in only a small percentage of carriers. This organism is designated as *Monilia X*, and is ordinarily of low virulence. The virulence on long cultivation is partially or completely lost, but may be recovered by passage through susceptible animals. When promptly injected, after recovery from patient with sprue, deaths generally result from mycotic septicaemia. Feeding tests with the freshly isolated organism from the patient ordinarily failed to kill animals, but when the virulence was raised by passage, it killed by this method of administration. Some of the animals died rapidly of a monilia septicaemia, others more slowly, probably from a toxin developed by a localization of the organisms in the intestinal tract; stomatitis has been observed, and also long continued severe diarrhoea following the feeding tests. Most of the tests were carried out on guinea pigs, although rabbits, monkeys, and the white rat were also used. The gross and microscopical findings of the autopsied animals are recorded. Noteworthy is the fact that the monilia, if attacking an internal organ, were seen as large colonies having the appearance of emboli. The intervening tissue spaces were generally free from the organism, thus radically differing from a bacterial septicaemia.

L. W. F.

Analysis of One Hundred and Thirty-Four Cases of Bacteriemia. M. Warren and W. W. Herrick. Am. Jour. Med. Sc., 1916, **151**, 556–577.

The author's report, as the title states, is an analysis of one hundred and thirty-four cases of bacteriemia observed during the past five years in the various services of a general hospital. An outline of the laboratory methods employed is given; also the bacteriological classification of the streptococcus group is considered. The cases are recorded under hospital case number, with age of the patient, the diagnosis, the maximum temperature, the leucocyte count and polynuclear percentage, the bacteriological blood findings, the treatment, and the result. Tables are given summarizing the authors' findings. Of especial interest to the bacteriologist is the table giving the General Summary, which follows:

General Summary

ORGANISM	NUMBER OF CASES	DIED	RECOVERED	IMPROVED	UNIMPROVED	DIED PER CENT
Str. hemolyticus......................	31	21	7	1	2	67.0
Str. viridans...........................	40	25	10	2	3	64.0
Str. mucosus...........................	1	1				100.0
Staph. aureus..........................	39	22	14	3		56.0
Staph. albus...........................	3	2	1			66.0
Pneumococcus..........................	10	6	2		2	60.0
B. coli.................................	6	4	2			66.0
B. influenzae...........................	2	2				100.0
Anaerobic streptococci.................	3	2	1			66.0
B. mucosus.............................	1	0	1			0
B. mallei...............................	2	2				100.0
B. alkaligenes..........................	1		1			0
Mixed infection........................	7	6	1			86.0
Str. hemolyticus and Staph. aureus.....	1	1				100.0
Str. hemolyticus and B. typhi..........	1	1				100.0
Str. viridans and Staph. aureus.........	1		1			0
Str. viridans and B. coli...............	1	1				100.0
Staph. aureus and B. coli..............	1	1				100.0
B. proteus and B. coli.................	1	1				100.0
B. influenzae and Staph. albus.........	1	1				100.0
All cases...............................	134	83				61.1

In the table giving the results of treatment are found twenty-five cases treated with vaccines with a mortality of 81 per cent, and four cases treated with serum, with a mortality of 75 per cent, which according to the authors shows "the futility of present measures of specific therapy of generalized infections."—L. W. F.

Tonsillar Endamebiasis and Thyroid Disturbances. J. S. EVANS, W. S. MIDDLETON AND A. J. SMITH. Am. Jour. Med. Sc., 1916, **151**, 210–222.

The authors discuss the various theories of the causation of endemic goitre, in particular, those more recently advanced, based on the view that the disease is of an infectious nature. From data obtained from the physical examination of a large number (1328) of men (University of Wisconsin) it was found that 27.2 per cent had thyroid involvement. Of these, 22.8 per cent showed infective cryptic tonsillar lesions; this was increased to 90 per cent when those having nasal lesions were included. These observations suggest a connection between the nasal and throat affections, and the thyroid involvement. Since *Entameba gingivalis* (Gros) apparently plays a rôle in the etiology of pyorrhea,

certain cases of chronic tonsilitis and systemic complications, the writer's attention was directed toward the organism. In thirty-four cases showing typically diseased tonsils, 97 per cent showed entamebae in the crypts. Of this group, sixteen individuals were treated by means of emetin hydrochloride with a disappearance of the amebae from the crypts in thirteen cases (81 per cent). Emetin was administered to twenty-three individuals, sixteen of whom showed an appreciable reduction in the bulk of the thyroid. The group included seven dysthyroid cases; six were benefited in varying degrees. The improvement under the treatment with emetin led the writers to believe that an indirect relationship existed, and they concluded that "A symbiosis of entamebae with appropriate bacteria, leading to the elaboration and absorption into the thyroid of selective thyrotoxic poisons, is at least conceivable in explanation of such relation." But they do not consider this to be an exclusive explanation of all goitres. No entamebae were found in the thyroid gland.—L. W. F.

THE BACTERIOLOGY OF THE BUBBLE FOUNTAIN[1]

DOROTHY F. PETTIBONE, FRANKLIN B. BOGART AND PAUL F. CLARK

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The public conscience has been aroused to the dangers of the common drinking cup as a possible means of spreading disease. Many state legislatures have passed laws prohibiting its use; other states have accomplished the same object by rulings of their state boards of health; and interstate passenger trains have been compelled to discard the common cup.

To meet this situation, three substitutes have been recommended; first, the individual cup carried and used by a single person, second, the single service paraffin cup, and third, the bubbling fountain. Expense and inconvenience have been large factors in preventing the general adoption of the first two methods. The bubble fountain, however, has met with cordial appreciation both on the part of the public and also in the hearts of health officials, and has been largely adopted in this country as the modern hygienic method of publicly providing for all comers drinking water free from possible contamination.

But is the bubble fountain as constructed at present free from danger? Have we not seized too readily upon an obvious improvement over the common drinking cup without adequate experimental basis?

Our attention was drawn to the bubble fountain as a possible source of danger during an epidemic of streptococcus tonsilitis which occurred in the fall of 1914 in one of the women's dormitories at the University of Wisconsin. Within a week's time, fifty cases were reported in this building. In studying the situation, it was noted that in this building the water pressure was so low that it was scarcely possible to drink from the bubble foun-

[1] Presented at seventeenth annual meeting of the Society of American Bacteriologists, Urbana, Ill., December 29, 1915.

tains in the hall without touching the metal portions with the lips.

An examination of these fountains showed them to be heavily contaminated with streptococci. Positive results were obtained from the surface of the fountain, from the inside, and from samples of the water. In fact, the swab from the inside of the bubble fountain on the third floor, where the greatest number of cases were located, gave an almost pure culture of streptococci. Several examinations of the city water supplying the fountains showed, on the other hand, no evidence of these organisms. The sanitary character of the city water supply is excellent, the water being obtained from deep wells in the Potsdam sandstone which underlies all southern Wisconsin.

Owing to a laboratory accident the identity of the organisms found in the bubble fountain and those isolated from the cases of tonsilitis was not established; and there were other cases of streptococcus tonsilitis in the town and also scattered cases throughout the University at the time. It seems a fair presumption, nevertheless, that these heavily contaminated fountains were a factor in spreading the infection within the dormitory.

A subsequent investigation of all the other bubble fountains in the University yielded surprising results. In examining the fountains, the following observations were made: type of fountain, height of column, bacterial count of the water on litmus lactose agar and action in glucose broth fermentation tubes which were subsequently examined for streptococci by the hanging drop method and by Gram stained smears. In addition, sterile throat swabs were rubbed thoroughly over the top of the fountain and as far into the bulb as possible. These swabs were placed in glucose broth which also was examined for streptococci by the methods mentioned above. The swabbings proved to be the most fruitful source of positive results. Only in a few instances did the amount of water tested prove to contain streptococci. Forty-three out of seventy-seven bubble fountains (55.8 per cent) were found contaminated with Gram positive streptococci which frequently occurred in long chains.

Many different types of bubble fountains are in use in the

University ranging from a plain rusty half-inch iron pipe with constant flow, in some remote corners of the campus, to the latest porcelain device with automatic shutoff in more prominent situations.   In general the simpler fountains such as the rusty pipe were not infected, whereas the complex fountains with intermittent flow and those with crevices and crannies between the inner and outer portions were the worst offenders, although presumably the location of the fountain was a factor in this result. In fact it is worthy of note in connection with the epidemic in the women's dormitory that streptococci were found most often in those buildings frequented by the women students while the Law Building, Engineering Building, Armory, etc., were quite as uniformly free from such contamination.

To control these rather surprising results a second survey of the fountains was made about a year later, at a time when there was no unusual number of upper respiratory infections, but following a period of unusual incidence of "grippe" both in the University and throughout the country.   Samples of water and swabbings were taken from fifty fountains, excluding many of those less commonly used.   The earlier results were completely confirmed, as Gram positive streptococci were found in 35 out of the 50, 70 per cent of the total.

The streptococci found are somewhat difficult to isolate, and readily overgrown by the common water organisms, developing well in glucose broth but not on other media.   They are only slightly virulent to experimental animals.   Further studies of these strains are being carried on.

As a further control a Berkefeld filter was attached to a city water faucet and the water allowed to flow for one week.   During this period it was estimated that about 3300 liters passed through the filter and yet smears and cultures made from the deposit on the filter showed not only no streptococci but no Gram positive organisms present.   This control was repeated.

In order to study the factors involved in the contamination of the fountains, a series of tests were performed on an experimental bubbler in the laboratory.   This particular fountain is one of an ordinary continuous flow type, the bubble being fed

through four perforations in the top of a cylinder which screws into the center of a hollow metal bulb. Uniform suspensions of *B. prodigiosus* were made by emulsifying each of two twenty-four hour cultures of the organism in 1 cc. of salt solution. This emulsion (2 cc.) was then dropped slowly from a pipette into the top of the bubble, care being observed not to touch the metal portions of the fountain. Ten cubic centimeter samples of the water were then taken at frequent intervals and plated in triplicate on agar, 1 cc. to each plate. After incubation the plates were allowed to stand at room temperature for several days in order to allow the *B. prodigiosus* colonies to develop their characteristic red pigment. Red colonies and total colonies were then counted. In all cases control samples of the water were taken before introducing the organisms. In a total of over 100 control plates, only one red pigment producer was found. This formed colonies quite different from *B. prodigiosus* colonies macroscopically and could never be mistaken for that organism. Between each two experiments the bubble fountain was removed, washed and sterilized.

The experiments were repeated several times with the column of water at different heights, varying from about 3 to 4 mm. (approximately the height of column prevailing in the women's dormitory at the time of the epidemic) to 10 cm., a column much higher than we have ever observed in use. The results obtained with the column at each level were somewhat variable. So many factors are involved that this is to be expected. The relatively small amount of water sampled as compared with the volume flowing during the period of sampling, the variation in the column caused by variation in water pressure, chance conditions such as the position of the pipettes both in introducing the organisms and in withdrawing the samples, together with unknown factors, must all play a part in the results observed.

In brief, when the bubble is extremely low (3 to 4 mm.) some of the introduced organisms are found in repeated experiments for as long as 135 minutes. With a column 2 to 3 cm. high, the average height of the jets in the University, *B. prodigiosus* organisms are retained frequently for 10 to 20 minutes and have

been found for as long as 90 minutes. A column 7.5 cm. in height has shown the presence of *B. prodigiosus* for 20 minutes although commonly no organisms have been found for more than 2 minutes. Even with the column 10 cm. high, *B. prodigiosus* has always been found in the plates poured one or two minutes after the introduction of the suspension.

Another type of experiment was tried more nearly approximating the ordinary use of the bubble fountain. The writers washed out their mouths with suspensions of *B. prodigiosus* and while the lips were still moist drank from a column 2 to 3 cm. in height, exercising due care not to touch the metal portions of the fountain. Samples of water were taken as before and plated. In one instance the organisms were retained for 135 minutes, and the average was 25 minutes.

The following tables show the results of sample tests taken at random from those made with the experimental fountain:

TABLE 1

Column 2 cm. high. Suspension of B. prodigiosus introduced by pipette, two twenty-four hours cultures 1 cc. salt solution to each culture

BACTERIAL COUNT	B. PRODIGIOSUS COUNT	TIME AFTER INTRODUCING ORGANISMS IN MINUTES
58	0	Controls
52	0	Controls
56	0	Controls
58	0	5
106	0	10
52	9	15
72	0	20
44	7	25
64	3	30
104	0	40
48	0	50
39	0	60
104	7	70
58	0	80
59	0	90
62	0	100
69	0	110

TABLE 2

Column 2 cm. high. Suspension of B. prodigiosus introduced as in table 1

BACTERIAL COUNT	B. PRODIGIOSUS COUNT	TIME AFTER INTRODUCING ORGANISMS IN MINUTES
160	0	Controls
130	0	Controls
142	0	Controls
58	13	5
136	4	10
52	1	15
72	13	20
64	2	25
149	21	30
104	35	45
104	5	60
61	1	75
95	2	90
95	0	105

TABLE 3
Column 2 cm. high   Suspension of B. *prodigiosus introduced as in table 1*

BACTERIAL COUNT	B. PRODIGIOSUS COUNT	TIME AFTER INTRODUCING ORGANISMS IN MINUTES
Not made	0	⎫
	0	⎬ Controls
	0	⎭
	5	1
	1	2
	3	3
	0	4
	1	5
	1	7
	1	10
	0	15
	0	20
	0	25
	0	30
	0	40
	0	50
	0	60
	0	70
	0	80
	0	90

TABLE 4
Column 3 cm. high.   Suspension of B. *prodigiosus introduced as in table 1*

BACTERIAL COUNT	B. PRODIGIOSUS COUNT	TIME AFTER INTRODUCING ORGANISMS IN MINUTES
Not made	0	⎫
	0	⎬ Controls
	0	⎭
	7	1
	1	2
	1	3
	1	4
	0	5
	0	7
	193	10
	0	15
	1	20
	0	25
	0	30
	0	40
	0	50
	0	60

These tables show that the efficiency of a bubble fountain depends partly upon the height of the jet thrown.   But even at the maximum height used, 4 inches, which is higher than practicable, some organisms are still retained in the column for about 2 minutes.   Considering also the fact that when the organisms were introduced on the moist lips, a very small number were added as compared with the millions introduced in the turbid suspension by means of the pipette, it is possible that the saliva of the mouth may be a factor in causing the bacteria to remain a longer time than would otherwise be the case.

How shall we explain these facts and how shall the bubble fountain be made safe for public use?

Our conception of the problem is that the organisms dance in the column of water much as the toy ball dances on top of the fountain in the garden.   Most of the organisms introduced are

TABLE 5

Column 10 cm. high.   Suspension of B.
prodigiosus introduced as in table 1

BACTERIAL COUNT	B. PRODIGIOSUS COUNT	TIME AFTER INTRODUCING ORGANISMS IN MINUTES
12	0	
20	0	
18	0	
20	0	Controls
14	0	
18	0	
15	2	1
12	1	2
8	0	3
10	0	4
6	0	6
12	0	8
9	0	10
15	0	15
18	0	20
8	0	30

TABLE 6

Column 2 cm. high.   B. prodigiosus
introduced by rinsing mouth with
suspension and then drinking while
lips were still moist.

BACTERIAL COUNT	B. PRODIGIOSUS COUNT	TIME AFTER INTRODUCING ORGANISMS IN MINUTES
Not made	0	
	0	Controls
	0	
		Drank from fountain
	0	1
	1	2
	1	3
	2	4
	1	5
	0	7
	0	10
	0	15
	1	20
	0	25
	0	30
	0	40
	0	50
	0	60
	0	70
	0	80

flushed off at once, whereas others "dance" for varying periods of time until they finally fall outside the column and disappear in the waste pipe.   Doubtless other factors are concerned, such as variation in pressure due to excessive use elsewhere on the same line and subsequent cessation of this unusual consumption of water.   Indeed a control series of plates of water samples taken at five minute intervals from a bubble fountain shows wide variation in bacterial count due partly to variation in pressure. When the pressure is suddenly increased the pipes are vigorously flushed and the bacterial count is higher.   Keith[2] (2) has suggested a modified form of fountain which automatically regu-

[2] Keith, S. C., Jr.   Amer. Jour. of Public Hygiene, 1910, **20**, 163.

lates the flow so that regardless of pressure changes, the height of the jet will remain constant.

But why retain a vertical column of water when it is obviously the upward direction of flow which more than any other factor renders the bubble fountain unsafe? A fountain (see fig. 1) was constructed with a simple tube, having no crevices or complications placed at an angle of about 55 degrees from the vertical.[3]
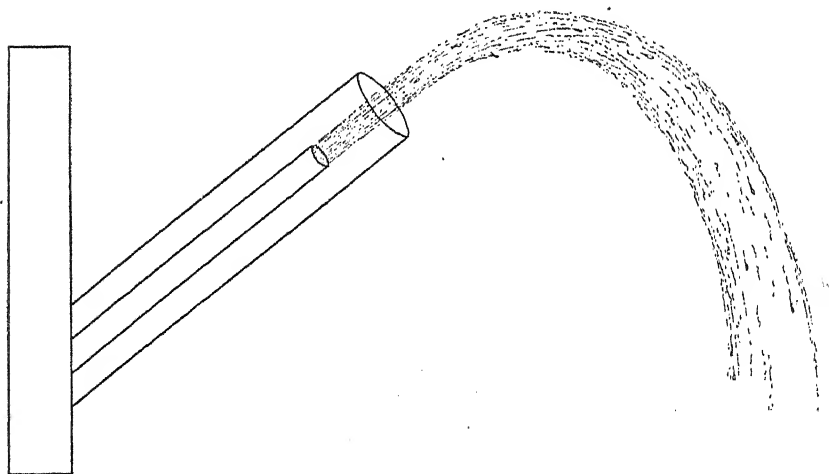
FIG. 1. SIMPLE TYPE OF FOUNTAIN WHICH SHOULD BE GENERALLY ADOPTED. NO CONTAMINATING ORGANISMS ARE RETAINED IN THE JET OF WATER

Even with a minimal stream flowing from this type of fountain, the introduced organisms were all immediately washed away and never did we find a single *B. prodigiosus* colony even on the plates poured immediately after the introduction of the organisms.

This simple type of fountain with an adequate collar guard to prevent possible contact with the orifice would, we believe, solve the problem and furnish water uncontaminated by human lips even to a constant succession of thirsty persons.

[3] Later experiments have shown that a tube erected at an angle of 15 degrees from the vertical is adequate to prevent organisms from "dancing" on the column of water. This makes the construction of a safe bubble fountain much more practicable.

TABLE 7

New model fountain with a small stream of water issuing from a simple tube at an angle of 50 degrees from the vertical. Suspension B. prodigiosus introduced as in table 1

BACTERIAL COUNT	B. PRODIGIOSUS COUNT	TIME AFTER INTRODUCING ORGANISMS
Not made	0	
	0	Controls
	0	
		seconds
	0	2
	0	5
	0	10
	0	15
	0	20
	0	25
	0	30
	0	35
	0	40
	0	45
	0	50
	0	55
	0	60
		minutes
	0	1
	0	2
	0	3
	0	4
	0	5
	0	7
	0	10
	0	15
	0	20
	0	25
	0	30
	0	40
	0	50
	0	60

CONCLUSIONS

1. During an epidemic of streptococcus tonsilitis in a woman's dormitory at the University of Wisconsin, streptococci were found in the bubble fountains in this building and in the water issuing from these fountains.

2. The city water supply was at the time, and has been, excellent in its sanitary character. It is obtained from the underlying Potsdam sandstone. No streptococci were found on a Berkefeld filter through which water had been flowing continuously for one week.

3. Presumably the bubble fountains were a factor in transmitting the disease.

4. A survey of all the fountains of the University showed the presence of streptococci in over 50 per cent of the total number. The streptococci varied in abundance from a few chains to an almost pure culture obtained by swabbings from the fountains in the women's dormitory.

5. In an experimental bubble fountain, *B. prodigiosus* when introduced either by means of a pipette or by the moistened lips remained in the water from 2 to 135 minutes depending partly on the height of the "bubble."

6. Most of the organisms are flushed away but some remain dancing in the column much as a ball dances on the garden fountain even though the bubble be increased to the impractical height of 4 inches.

7. To avoid the difficulty always present in the vertical column, a simple fountain with a tube at an angle of 50 degrees from the vertical was constructed. *B. prodigiosus* was never found in the plates from this type of fountain even when samples were taken immediately after the introduction of the organisms.

8. We believe that this type of fountain should be generally adopted. Its simplicity, low cost of construction and freedom from lurking danger should recommend it to all.

THE ADVANTAGES OF A CARBOHYDRATE MEDIUM IN THE ROUTINE BACTERIAL EXAMINATION OF MILK

JAMES M. SHERMAN

From the Bacteriological Laboratories of the Pennsylvania State College and Agricultural Experiment Station

INTRODUCTION

The object of this paper is to direct the attention of laboratory workers to the advantages over plain agar of an agar containing a fermentable carbohydrate, for use in the bacteriological control of market milk. Some laboratories are probably aware of these facts, but since the standard methods for the bacterial analysis of milk prepared by the committee of the American Public Health Association[1] call for the use of plain agar, and since this medium is used in many of the largest laboratories making routine analyses at the present time,[2] it seems pertinent to submit a few data on these points.

METHODS

The bacterial counts reported herein were made on plain agar prepared according to the standard formula, and on lactose agar with the same ingredients plus 1 per cent of lactose. Liebig's beef extract and Witte's peptone were used. The media were adjusted to a reaction of 0.5 per cent normal acid to phenolphthalein, which reaction is, according to the results obtained in this laboratory,[3] better than a higher acidity.

In the determination of bacterial numbers triplicate plates were made of each dilution, and the counts were made after

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[1] Meetings of Am. Pub. Health Assn., Rochester, N. Y., September, 1915.

[2] U. S. Pub. Health Service, Public Health Reports, **30**: 2349–2394.

[3] Penn. Agr. Expt. Sta., Report for 1914–1915.

forty-eight hours incubation at 37°C. A hand glass with a magnification of three and one-half diameters was used in counting the plates made with plain agar, but the lactose agar plates were counted with the naked eye.

For the comparison of the size of colonies which developed on plain and lactose agars the plates were incubated forty-eight hours at 37°C. and measurements made by means of a compound microscope and eyepiece micrometer which gave a magnification of fifty diameters.

QUANTITATIVE BACTERIAL ANALYSIS

In table 1 are given the results obtained from eighteen samples of raw market milk when plated on plain and on lactose agars. Among these milks were samples representing all kinds of milk from a very good to a very low-grade type.

TABLE 1

Number of bacteria on plain and lactose agars

Raw Milk

SAMPLE NUMBER	NUMBER OF BACTERIA PER CUBIC CENTIMETER		INCREASE IN FAVOR OF LACTOSE AGAR
	Plain agar	Lactose agar	
			per cent
1	817	1,017	25
2	1,340	2,070	55
3	1,630	2,000	23
4	3,230	3,530	9
5	7,330	7,330	0
6	8,000	11,300	41
7	8,900	12,100	36
8	9,030	8,970	−1
9	11,800	11,170	−5
10	186,000	610,000	228
11	192,000	278,000	45
12	260,000	361,000	39
13	369,000	463,000	26
14	53,700,000	109,000,000	103
15	216,300,000	346,600,000	60
16	421,300,000	640,000,000	52
17	576,000,000	1,216,000,000	111
18	1,036,000,000	1,312,000,000	27

The data given in this table indicate that lactose agar is of considerably more value than plain agar for the quantitative bacterial analysis of milk. Of the eighteen samples examined, fifteen gave higher counts on lactose agar; with one sample there was no difference, while two samples gave counts which showed a negligible advantage in favor of plain agar. If we consider the experimental error as about 20 per cent, and discard all differences of less than that amount, it will be seen that fourteen, or 78 per cent, gave increased counts on the lactose agar, while none of the samples showed a similar increase in favor of plain agar. Taking all eighteen samples we find an average increase of 43 per cent in the counts obtained with lactose agar.

The results reported in table 1 are all from samples of raw milk. In table 2 will be found the data obtained from six sam-

TABLE 2

Number of bacteria on plain and lactose agars

Pasteurized Milk

SAMPLE NUMBER	NUMBER OF BACTERIA PER CUBIC CENTIMETER		INCREASE IN FAVOR OF LACTOSE AGAR
	Plain agar	Lactose agar	
			per cent
1	7,600	25,300	233
2	6,500	42,500	554
3	6,000	69,000	1050
4	18,100	43,600	141
5	23,800	58,000	144
6	72,000	177,000	146

ples of pasteurized milk. Three of the samples were pasteurized by the holder method at 140°C. for twenty minutes, while the other three were treated by the flash method at 180°C. The milks were plated within an hour after pasteurization. The results are very striking and demonstrate the superiority of lactose agar over plain agar for the examination of freshly pasteurized milk.

THE SIZE OF COLONIES ON PLAIN AND LACTOSE AGARS

Although the difference in the counts obtained is sufficient reason for advocating the use of a carbohydrate medium for the

bacterial examination of milk, probably the strongest point in its favor is the difference in size of colonies which develop on the two media. The colonies on lactose agar are always considerably larger than those on plain agar, this being especially true of the acid-forming organisms. Photographs were taken of the petri dish cultures obtained and reproductions of some typical plates are appended to this paper. As was stated before, the plain agar plates were counted with the aid of a hand lens as prescribed by the standard methods for the bacterial analysis of milk, whereas the plates containing lactose agar were counted with the naked eye. This practice was followed because the colonies on lactose agar were of sufficient size to make the use of a lens unnecessary. Many trials have failed to reveal colonies on lactose agar under a hand lens which were not visible to the unaided eye. This, however, might not be true in the case of a worker with defective eyesight.

In order to get some definite information on the difference in size of colonies on the two media, pure cultures of B. lactis-acidi— which is usually the predominating organism in ordinary market milk—and a streptoccus—which is usually the predominating organism in milk of a certified grade—were plated out on plain and lactose agars, and the colonies which developed were measured after incubation at 37°C. for forty-eight hours. Only plates which contained less than one hundred colonies were used so as to eliminate as far as possible the factor of inhibition. The ten largest colonies on each plate were measured. These data are given in table 3.

These differences in size of colonies are presented graphically in figures 1, 2, and 3.

DISCUSSION

It should be understood that the data here presented are intended to apply only to the routine examination of milk for which a short period of incubation is used. Although the few data at hand indicate that quite similar results are obtained with lower temperatures and longer periods of incubation, it is not within the province of this paper to draw such conclusions.

TABLE 3

Size of colonies on plain and lactose agars

| NUMBER OF COLONY | DIAMETER OF COLONIES IN MICRONS | | | | | |
| | *B. lactis-acidi* (Culture A) | | *B. lactis-acidi* (Culture B) | | Streptococcus | |
	Plain	Lactose	Plain	Lactose	Plain	Lactose
1	50	660	340	560	220	460
2	80	720	280	600	230	440
3	60	540	320	700	220	500
4	50	520	260	1300	220	480
5	60	500	240	600	240	510
6	70	640	220	640	210	580
7	80	820	300	590	240	600
8	60	540	300	1140	220	570
9	60	620	300	1080 ·	260	580
10	70	580	280	1120	220	640
Average............	64	614	284	833	228	536
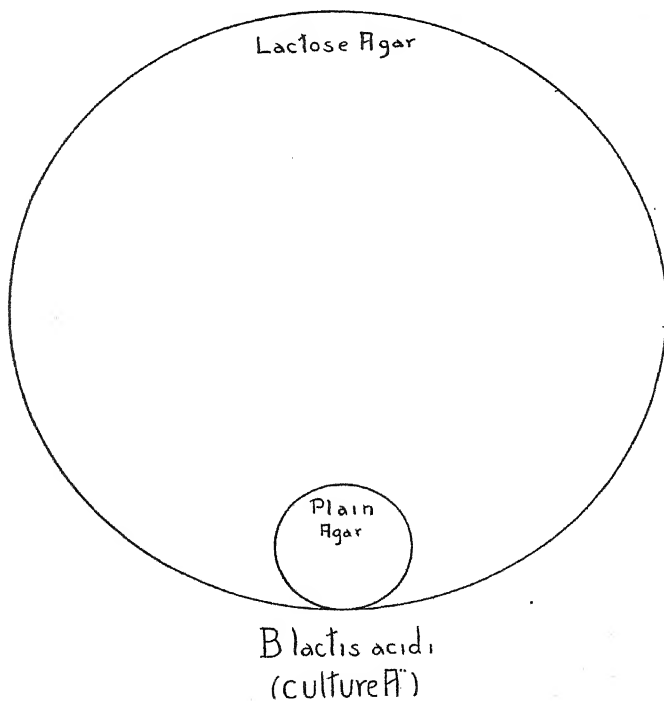
Fɪɢ. 1. The relative size of colonies of *B. lactis-acidi* grown on plain and lactose agars. Plates were incubated at 37°C. for forty-eight hours. Graphs are plotted from the average of ten colonies from each medium.

Lactose agar was used in all of the tests herein reported, but from the results of a number of comparisons it may be said that glucose is apparently just as efficient as is lactose, both as to number and size of colonies. According to the work of Heine-mann and Glenn[4] glucose and lactose agars are of equal value
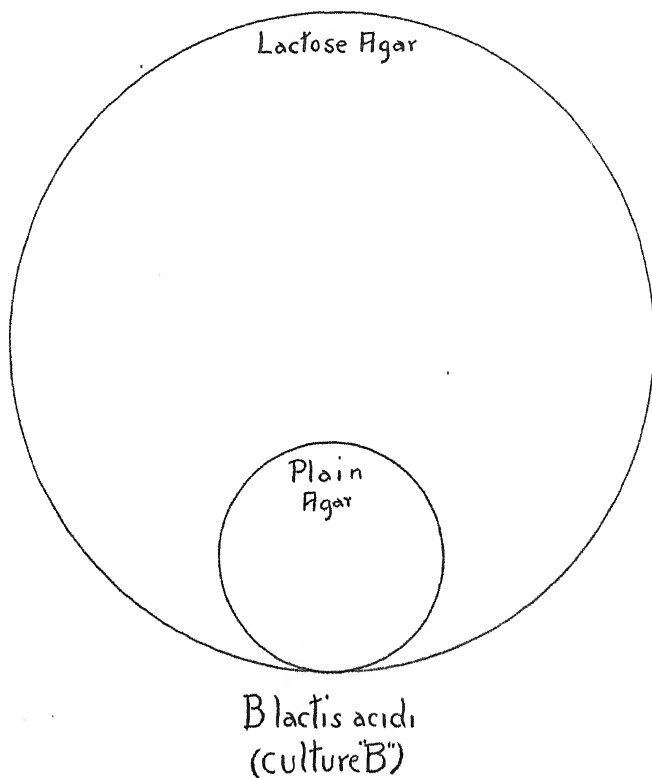
FIG. 2. Same as figure 1 except a different strain of *B. lactis-acidi* was used.

for the quantitative bacterial analysis of milk, and since glu-cose is cheaper it should, perhaps, be recommended as the stand-ard for routine work. A limited number of trials have also

[4] Jour. Infect. Dis., **5**: 412–420.

indicated that the amount of carbohydrate may be reduced to 0.1 per cent without impairing the value of the medium.

Another advantage of a medium containing a carbohydrate over plain agar is that the former is of considerable value in differentiating the types of organisms on the plates. The colonies of acid-producing bacteria on sugar agar are surrounded by
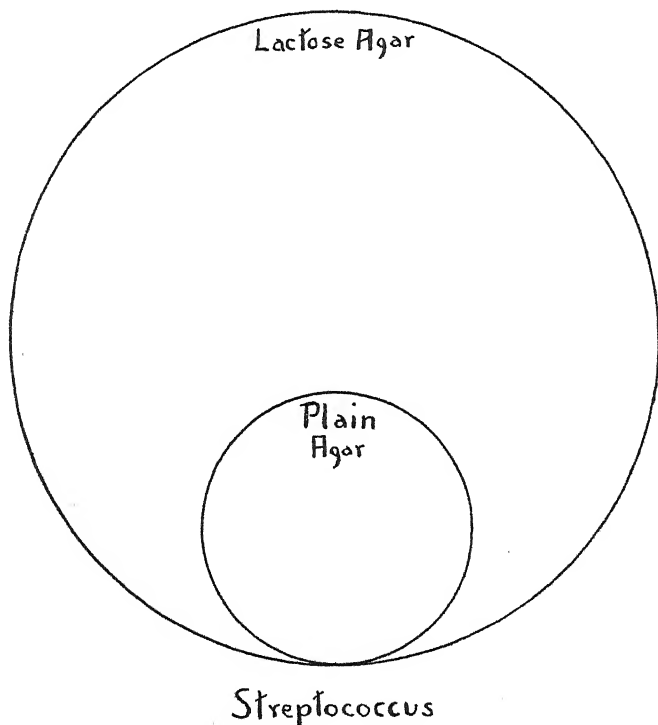
FIG. 3. Same as figure 1 except a culture of a streptococcus was used.

a precipitate of protein matter, thus giving the typical hazy edge which is so characteristic of organisms of the *B. lactisacidi* group. On agar made without clarification with egg albumen, and which is consequently more opaque, the peptonizing bacteria may be quite readily identified by means of the clear

halo which surrounds their colonies. This differentiation is much clearer on plates containing lactose or glucose agar than on those with plain agar, as the opacity of the medium is very much increased in the presence of a fermentable carbohydrate, due to the growth of acid-producing organisms. In fact, unclarified lactose agar gives, in our hands, nearly as clear a differentiation of bacterial types as does the special casein agar devised by Ayers.[5] These points are well illustrated by the following photographs.

SUMMARY

Data are presented which it is thought warrant the recommendation that agar containing glucose or lactose be adopted as the standard medium for the routine bacteriological analysis of milk.

The advantages of a carbohydrate medium over plain agar may be summed up in the following points:

1. A higher count is obtained.

2. The colonies grow larger and more rapidly which greatly facilitates the counting of plates.

3. It is of some value as a differential medium.

[5] U. S. Bureau of Animal Industry, Report for 1911.
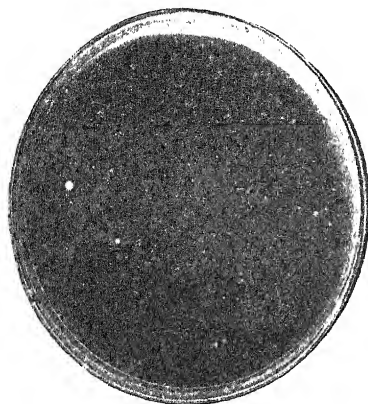
PLAIN AGAR   LACTOSE AGAR

Milk "a."   Dilution of 1/100 on plain and lactose agars.   Incubated at 37°C. for forty-eight hours.
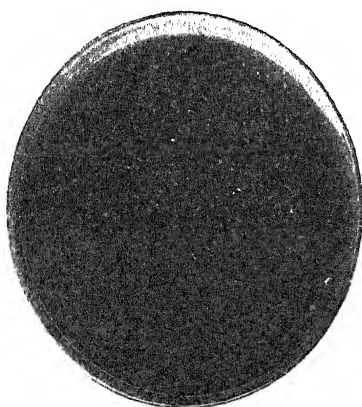
PLAIN AGAR   LACTOSE AGAR
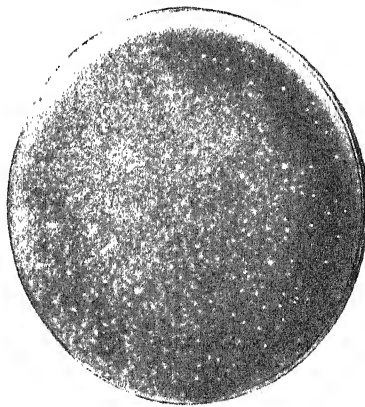
Milk "b."   Dilution of 1/1000 on plain and lactose agars.   Incubated at 37°C. for forty-eight hours.

(Sherman: Advantages of a Carbohydrate Medium)
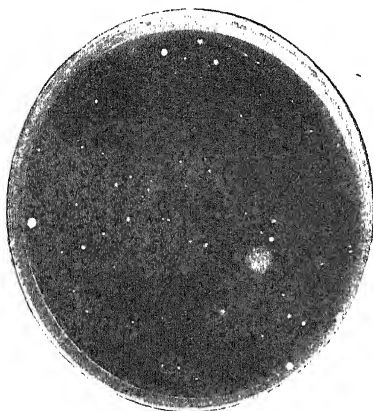
PLAIN AGAR   LACTOSE AGAR
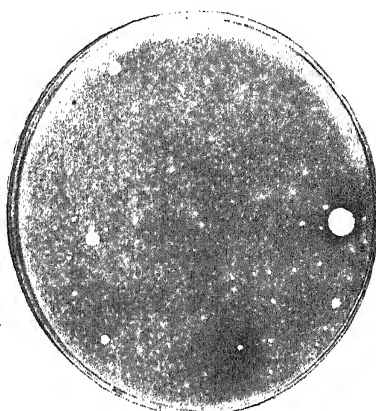
Milk "c."   Dilution of 1/100 on plain and lactose agars.   Incubated at 30°C. for six days.
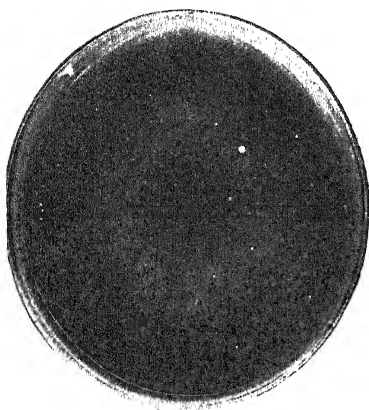
PLAIN AGAR   LACTOSE AGAR
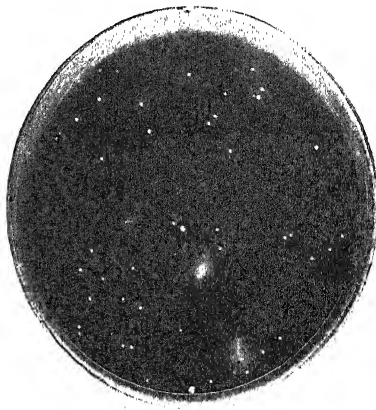
Milk "d."   Dilution of 1/10,000 on plain and lactose agars.   Incubated at 37°C. for twenty-four hours.

(Sherman: Advantages of a Carbohydrate Medium.)

ON A SPECIES OF TREPONEMA FOUND IN RABBITS

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In the course of many attempts to isolate new strains of *Treponema pallidum* from luetic rabbits, the writers have encountered a spiral microörganism which has appeared on six different occasions in tubes prepared from the tissues of five different strains of rabbit syphilis. On first observation of this microörganism it seemed difficult to decide whether it represented a distinct form, or a morphological mutation of *Treponema pallidum*. Subsequent studies, however, have led us to feel reasonably sure that the organism has no relation to *T. pallidum*, but represents a distinct species present in the rabbit tissue, which grows in the tubes as prepared by the Noguchi method. It is a curious fact that although innumerable tubes have been prepared by the same method, in the course of the last three years, with normal rabbit kidney for transplantation of pure cultures of *Treponema pallidum* (both Noguchi's strains and our own), the microörganism concerning which we wish to report has never appeared in such cultures but has been seen only in those implanted with the syphilitic testicular tissue of diseased rabbits. We therefore believe it at least very unlikely that the organism had its origin in the normal rabbit kidney tissue which was used in both kinds of culture. Since we have not made more than a few dozen plants with normal rabbit testis, we can not decide whether the microörganism we are describing may be a parasite in normal rabbit testes, or whether its presence is incidental only to the abnormal conditions occurring in the luetic lesions. To determine this would probably require a great deal of experimentation and time (to say nothing of luck), since we have encountered the organism but six times in the course of three years during which many hundreds of tubes were planted and examined.

It was startling to find this organism growing only when syphilitic material had been planted, and then multiplying with sparseness and cultivated with difficulties much greater than those attending the eventual cultivation of the limited number of strains of *T. pallidum* successfully grown by various workers. However, the obvious suspicions as to its connection with the syphilitic lesions aroused by these, facts seemed easily refuted by the morphology of this treponema which is very different from that of both the virulent treponemata and of the cultivated *T. pallidum* described by Mühlens, Noguchi, and others, and studied for several years by us.

The microörganism is a very fine spiral with curves having the absolute regularity of a corkscrew in most of the individuals, with finely tapering ends, and varying in length from 2 to 10 or 20 rather shallow curves. Both short and long forms are from two to three times as thick as the *Treponema pallidum*, and in most individuals a definite double contour is visible. The average length varies from about one-half to three or four times that of the *Treponema pallidum* and occasional long forms are seen which extend completely across the dark field of a one-twelfth oil immersion lens. The curves are long and shallow.

What is most noticeable about these treponemata is their absolute rigidity and lack of any kind of motility. At first we took it for granted that the organisms were dead. However, when we found that subsequent generations in culture were equally immobile, it became evident that this was characteristic of the species.

The organism stains with great difficulty. Its contours appear faint and are often less distinct than those of the *Treponema pallidum* after 12 hours staining in dilute Giemsa. It can be demonstrated with the Loeffler flagella stain and by the Fontana method. We have so far failed to stain it with the ordinary dyes.

Cultivation is extremely difficult. As stated above, multiplication has appeared in ascitic agar tubes prepared with rabbits' kidney as in the Noguchi method. We have had three or four strains which have proceeded to the third or fourth generation only to be lost. At present we have a strain still growing

in the fourth generation.   The cultures in which it multiplied were sealed with oil, and at first we found the organism near the bottom of the tubes, about the kidney tissue.   Subsequent study, however, revealed that the most likely place to find them was about one inch from the top under the oil.   In such tubes the growth was sometimes indicated by a very faint haziness not seen without careful examination and usually not larger than the head of a pin.

In the material fished from such an area, small clumps of three, four, or five spirals may be seen under the dark field as depicted in the photographs.   We have not yet been able to purify this organism, a task of great difficulty because of its immobility.   We have not had a sufficiently profuse growth to attain success by the dilution methods, and the first appearance of the microörganism has so far .occurred only in contaminated tubes together with motile bacilli.   The continued cultivation of the microörganism has, as it were, hung on a thread, the growth being very sparse and taking about a month or two to develop at all.

We have withheld the report on this organism for many months because we have been unable to obtain it in pure culture.   Unless chance favors our efforts more than heretofore, it may take a year or two to achieve this, and perhaps we may lose and recover it several times before this result is obtained.   We venture to make this brief report now, therefore, since many investigators are studying the cultivation of *Treponema pallidum* from rabbits and we have not seen any mention of a microörganism of this kind, so easily differentiated morphologically from *T. pallidum*, yet appearing in rabbits in the same location and under the same conditions.

Knowledge of its occurrence and its limitation, so far in our experience, to cultures from syphilitic testis should be of value to others working in this field.   Should it eventually prove to be a hitherto undescribed species, we would suggest as a suitable name for it "*Treponema rigidum.*"
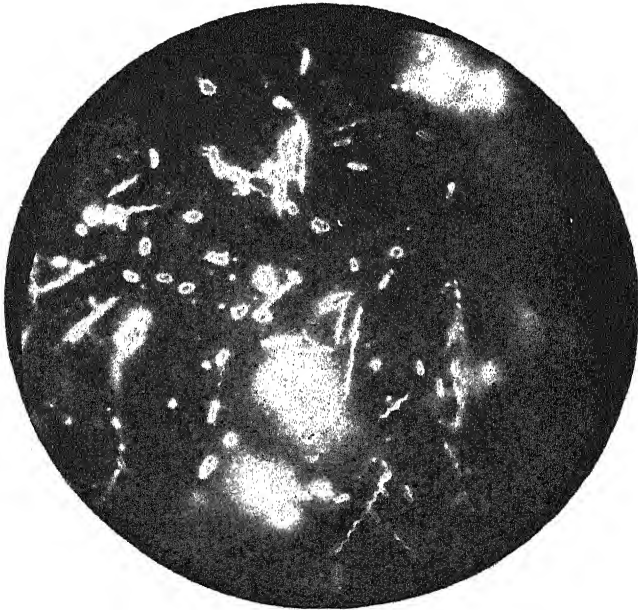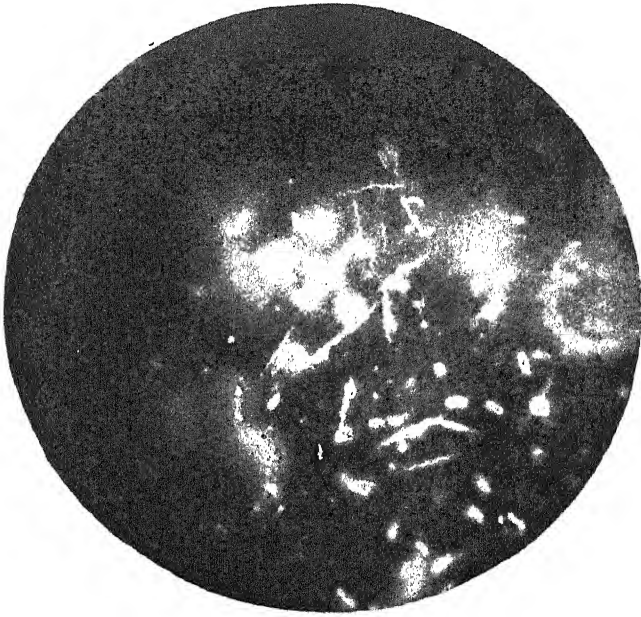
DARK FIELD PHOTO-MICROGRAPHS OF NON-MOTILE TREPONEMA FOUND
IN TESTES OF ENETIC RABBITS

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STUDIES ON AEROBIC SPORE-BEARING NON-PATHOGENIC BACTERIA

PART II

From the Laboratory of Hygiene and Bacteriology, Johns Hopkins University

SPORE-BEARING BACTERIA IN DUST

BY C. A. LAUBACH

Spore-bearing organisms from dust were obtained by rubbing moist sterile swabs over various dust-laden surfaces, transferring the material thus obtained to melted agar and then heating to 90°C. for fifteen minutes to destroy all non-sporulating bacteria. Plates were then poured in the usual way and different colonies selected for study and identification. In many instances the cultures had to be replated a number of times before the purity of the strain was established, so closely do the spores adhere to each other. In general the most prolific source of the spore-bearing organisms was dust which had lain undisturbed for long periods of time as in closets or on high shelves. Dust particles circulating in the air seemed relatively free from spore-bearing bacteria but an increase of these species was always noted with an increased velocity of the wind. Dust from moist surfaces allowed to dry down and from surfaces exposed to direct sunlight contained few spore-bearers. Numerous strains were obtained from the dust found on books. Some 312 cultures were studied and the types (as established in accord with results of the previous work on milk and on miscellaneous cultures) were found to be distributed as follows.

Bacillus cereus Frankland.. 93
Bacillus subtilis (Ehrenberg) Cohn................................ 71
Bacillus vulgatus (Flügge) Trevisan.............................. 46
(*Bacillus mesentericus vulgatus* Flügge.)
Bacillus megatherium De Bary..................................... 39

Bacillus petasites Gottheil...................................... 21
Bacillus mesentericus (Flügge) Migula........................... 17
(*Bacillus mesentericus fuscus* Flügge.)
Bacillus aterrimus Lehmann & Neumann....................... 8
(*Bacillus mesentericus niger* Lunt.)
Bacillus fusiformis Gottheil.................................... 4
Bacillus brevis Migula.. 2
Bacillus albolactus Migula...................................... 1
Bacillus terminalis Migula...................................... 1

In addition to these previously established types, on four occasions an organism was encountered giving the same reactions as the species discovered by Prausnitz in Flügge's laboratory and described by Flügge (1886) as *Bacillus ramosus liquefaciens*. This organism was correctly named *Bacillus prausnitzii* by Trevisan (1889). It is distinct from *Bacillus mycoides* of Flügge but the use of the term, "Würzelbacillus" and the name "Ramosus" for this latter organism by both Eisenberg (1891) and the Franklands (1894) with a coincident description of *Bacillus mycoides* as a distinct species by the latter has led to hopeless confusion. Our investigations show that the majority of organisms of this group, producing felted growths in the depths of agar, correspond in all particulars to *Bacillus mycoides* of Flügge which is probably identical with the "Würzelbacillus" and also with the *Bacillus ramosus* of both Eisenberg and the Franklands. The *Bacillus ramosus liquefaciens* of Flügge is a distinct species which we shall describe under its correct name *Bacillus prausnitzii* Trevisan (syn. *Bacillus ramosus liquefaciens* Flügge).

Two cultures were isolated which exhibited the morphology and general cultural characters of the members of the "mesentericus" group but produced an abundant yellow pigment. At first we were inclined to regard this organism as identical with the species described by Sternberg (1892) as *Bacillus subtilis similis*, but the morphology was so clearly that of the mesentericus type that it was deemed best to describe it as a new variety of *Bacillus mesentericus* to which the varietal name *flavus* is given. On one occasion a culture was obtained which seems to represent *Bacillus ruminatus* of Gottheil. It shows the peculiar porcelain-white growth on agar said by Gottheil to be the prin-

cipal feature distinguishing *B. ruminatus* from *Bacillus megatherium*, which it most closely resembles. The species described originally by Vogel (1897) as *Bacillus mesentericus panis viscosi I*, now known as *Bacillus panis* Migula was found once; and one isolation proved to be a new species belonging to the mycoides group to which we have given the name BACILLUS ADHAERENS. The above list of organisms found in dust must therefore be supplemented by the following types whose descriptions are given below in full.

Bacillus prausnitzii Trevisan...................................... 4
(*Bacillus ramosus liquefaciens* Flügge.)
Bacillus mesentericus variety *flavus* nov. var....................... 2
Bacillus ruminatus Gottheil...................................... 1
Bacillus panis Migula 1
(*Bacillus mesentericus panis viscosi* I Vogel.)
BACILLUS ADHAERENS, nov. sp........................... 1

Bacillus prausnitzii Trevisan

This organism was originally described as *Bacillus ramosus liquefaciens* by Flügge. It is sometimes regarded as identical with *Bacillus mycoides* but a culture obtained from the Kral collection in Vienna shows different reactions from those of *Bacillus mycoides*. Several cultures corresponding closely to the Kral culture were isolated from dust. The following description applies to the Kral culture and to our own isolations, as well.

Morphology. In young cultures 6 hours old on plain agar the organisms are homogeneous, have round ends when free and flattened ends when in juxtaposition. They generally occur in chains of 2 to 4 elements and resemble *Bacillus mycoides* in morphology. The single cells measure 0.625 to 0.75 by 3 to 5 microns. On glucose agar they are thicker measuring 0.75 to 1 by 3 to 5 microns. In older cultures, 24 hours, on both plain and glucose agar the protoplasm is converted into globular bodies which take the stain badly. These are especially abundant on glucose agar which also shows long and thick vegetative rods measuring 0.75 to 1 by 4 to 6 microns and peculiar washed-

out organisms which seem to be made up of a fine network or skein of filaments.   (Figures 53 and 54.)

Motility.   Active motility in young cultures.

Staining properties.   Gram-positive.

Spore formation.   Spores begin to form early appearing in 24 hours on plain and on glucose agar.   They are usually central, one to a cell, and are slightly wider than the vegetative rods. The organisms with spores retain their chain formation and later the free spores may also remain attached in chains.   The free spores are cylindrical and measure 0.75 to 1 by 1.5 to 2 microns.   As they lose their protoplasm they become oval and measure 0.625 to 0.75 by 1 to 1.25 microns.

Agar slant.   Profuse spreading dull growth consisting of fine interlacing filaments developing from the central line of inoculation as a rhizoid mass.   The early growth is extremely tenacious and extends deeply into the underlying agar.   Later the growth becomes finely granular and friable and can be scraped from the medium.   In general the appearance on agar is like that of a culture of *Bacillus mycoides.*

Agar stab.   Abundant growth along line of inoculation and spreading surface growth.

Agar colonies.   Colonies consist of profusely interlacing filaments spreading from opaque centers.   They are dull grayish and penetrate the agar, under the surface of which they grow in the medium.

Glucose litmus agar slant.   Scanty growth on the surface with a pronounced acid reaction which remains permanent.

Glucose litmus agar colonies.   Colonies much the same as those on plain agar but somewhat more profuse, with the filamentous character more pronounced.

Gelatin stab.   Progressive funnel-like liquefaction often complete within three days.

Gelatin colonies.   Colonies consist of profusely interlacing filaments spreading from opaque centers.   They are dull greyish and penetrate the gelatin, under the surface of which they grow as in agar.   Each colony is soon surrounded by a zone of liquid gelatin.

Broth.   Granular scum and flocculent growth which soon settles to the bottom.

Peptone.   Similar granular scum and flocculent sediment.

Potato.   Viscous yellowish-gray growth spreading profusely and rapidly over the whole medium.

Litmus milk.   Reaction highly acid within 24 hours and a firm coagulation within 48 hours.   Peptonization soon begins and proceeds very slowly eventually converting the coagulum to an amber-colored fluid.   Coagulation is more rapid in freshly isolated strains.

Blood serum.   Profuse moist dull interlacing or mycelioid growth.   No peptonization.

Fermentation tubes.   Glucose: a flocculent growth in the bowl and a granular scum.   Turbidity in closed arm.   Reaction acid.

Saccharose: a flocculent growth in the bowl with an acid reaction.   Arm usually clear.

Lactose: a similar flocculent growth with an acid reaction. Arm usually clear.

Thermal death point.   Spores survive 12 pounds in the autoclave but are killed by 15 pounds.   In the Arnold they survive 45 minutes but are destroyed by an hour's exposure.

Bacillus mesentericus variety *flavus* nov. var.

This is a new variety of *Bacillus mesentericus* to which the name *flavus* is given because of the abundant yellow pigment it produces.   We have encountered it repeatedly in dust and in soil.

Morphology.   Thin homogeneous rods with round ends, in young cultures on plain agar measuring 0.375 to 0.5 by 1.5 to 4 microns.   On glucose agar the organisms are a little thicker and longer, measuring 0.5 to 0.75 by 3 to 5 microns.   They often grow in long threads measuring 9 to 12 microns in length. Shadow forms are formed early both on plain and on glucose agar. In old cultures especially on glucose agar the long forms tend to curve.   (Figures 55, 56 and 57.)

Motility.   Actively motile in young cultures.

Staining properties.   Gram-positive.

Spore formation.   Spores begin to form within 24 hours. By the end of the 2nd day the spores are abundant. They arise in the center or towards one end of the organism and bulge the rod but little. The free spores retain rims of protoplasm often greater in quantity at one end than at the other. They resemble the mesentericus spores and measure usually 0.625 by 0.75 to 1.5 microns. As they mature they lose their rims of protoplasm, become more oval and measure 0.5 to 0.625 by 0.75 to 0.875 microns.

Agar slant.   Moist, smooth, non-spreading lemon-yellow growth. The lemon-yellow color becomes more pronounced with age.

Agar stab.   Profuse granular growth along line of stab with slight irregular outgrowths.

Agar colonies.   The colonies are smooth, moist, round, with no tendency to spread, glistening and raised, lemon-yellow in color. There is a definite opaque yellow center while the periphery is translucent and shell-like. Deep colonies have a tendency to spread and become faintly iridescent.

Glucose litmus agar slant.   A very scant, moist, granular and slightly yellow growth is produced with a slight acid reaction.

Glucose litmus agar colonies.   Colonies similar to those on agar but less profuse. Reaction of medium acid.

Gelatin stab.   A very slow liquefaction is produced along the line of stab and a slight yellow tinge is imparted to the medium.

Gelatin colonies.   The colonies rest in slight depressions caused by slow liquefaction. They are dark yellow in color, moist, round and smooth. Under the low power of the microscope each colony is seen to be composed of concentric circles of varying densities with lobate edges.

Broth.   A slight turbidity appears after a considerable period. The medium clears by sedimentation and the sediment has a slight yellow tinge.

Peptone.   A slight turbidity, somewhat less than in broth occurs. This soon settles to the bottom.

Potato.   No visible growth.

Litmus milk. No change in reaction even after a long period of time.

Blood serum. A scant, moist, smooth, glistening and yellowish growth is produced. No solution of the medium occurs.

Fermentation tubes. Glucose: a slight turbid growth occurs in the bowl with the production of a slight acidity.

Saccharose: the same appearance. No acid.

Lactose: slight turbidity. No acid.

Thermal death point. Spores survive 18 pounds pressure in the autoclave but are destroyed by 20 pounds. They survive one hours steaming in the Arnold.

Bacillus ruminatus Gottheil 1901

The type "ruminatus" was first described by Gottheil (1901). In morphology and cultural characters it closely resembles *Bacillus megatherium* from which it is distinguished by its porcelain-white growth, particularly in young cultures. The present description applies to an organism found in dust and subsequently in water which corresponds to Gottheil's original description but not however to that given by Chester. We believe that it may properly be called *Bacillus ruminatus*.

Morphology. Homogeneous rods with rounded ends measuring 0.625 to 0.75 by 2.5 to 4 microns in young cultures on plain agar. On glucose agar they are distinctly thicker and longer measuring 0.75 to 1.125 by 2.25 to 5 microns. Rarely, long forms are found on this medium measuring 8 to 10 microns in length. Shadow or washed out forms are common on both plain and glucose agar measuring 1.025 to 1.5 by 3 to 5 microns. Organisms often appear in short chains. (Figures 58 and 59.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed early, often in 48 hours, especially when the strains are first isolated. They are central or slightly ex-centric and on sporulation swell or bulge the organisms from which they spring. They measure usually 0.625 by 1.25 to 1.5 microns. Some spores are however somewhat smaller and more oval.

Agar slant. Moderate growth along line of inoculation forming a definite ridge. There is a tendency for the growth to spread to either side and the opacity of the growth decreases towards the periphery which is translucent. The growth is glistening, raised, moist and has a pure white color which becomes more apparent with age.

Agar stab. Growth along the line of inoculation distinctly granular.

Agar colonies. The colonies are very characteristic. Some are round and regular while others show a considerable tendency to spread. They are moist, opaque, raised, glistening and white. The majority show dense centers surrounded by thin areas which are in turn surrounded by translucent shell-like peripheries.

Glucose litmus agar slant. The growth is similar to that on agar although somewhat variable as to amount. The distinct white coloration is also evident. A definite and permanent acidity is produced.

Glucose litmus agar colonies. The colonies correspond closely to those on agar but are less profuse.

Gelatin stab. A fairly rapid progressive cone-like liquefaction occurs.

Gelatin colonies. The colonies rest in a cup-like excavation caused by liquefaction. They are definitely circumscribed and have an opaque center which is surrounded by a less dense grayish area. This in turn is enclosed by a more dense grayish ring. Outside this ring the opacity decreases towards the periphery.

Broth. A fine fragile pellicle is formed with some turbidity. The medium clears by sedimentation.

Peptone. Similar scum but less marked turbidity.

Potato. Cream-white moist profuse growth developing in 24 to 48 hours.

Litmus milk. Within 24 hours a slight acid reaction occurs and the milk often shows a distinct thickening at the bottom. The coagulation is not definite however and peptonization begins usually within 48 hours. As it advances a clear zone of amber-colored fluid is found at the upper part of the milk tube. This gradually increases until all the milk is peptonized.

Blood serum. Fairly profuse moist glistening smooth whitish growth. Some softening of the serum but no definite liquefaction.

Fermentation tubes. Glucose: a slight turbidity in the bowl and neck with a reduction of the litmus in the closed arm. Reaction acid.

Saccharose: a similar growth with an acid reaction.

Lactose: only a slight turbidity develops with an alkaline reaction.

Thermal death point. The spores survive 20 pounds pressure in the autoclave but are destroyed by 22 pounds. They survive one hours steaming in the Arnold.

Bacillus panis Migula 1900

This organism was originally described by Vogel (1897) as *Bacillus mesentericus panis viscosi I.* It has been found in Baltimore, but once, in dust.

Morphology. When first isolated this organism showed only encapsulated forms, the capsules staining readily with gentian violet. As the organism was cultivated on artificial media in the laboratory it lost its capacity of forming easily-stained capsules but continued to manufacture a quantity of viscous material which gave a characteristic appearance to the cultures. In young cultures on plain agar, 6 to 24 hours old, the organisms are small and homogeneous with round to flattened ends, measuring 0.375 to 0.5 by 1.5 to 3 microns. They show no appreciable difference in thickness on glucose agar, but tend to show long forms measuring 5 to 6 microns in length. Occasional shadow forms are seen measuring 0.75 to 1 by 1.5 to 4 microns. (Figures 60, 61, and 62.)

Motility. No motility has thus far been demonstrated.

Staining properties. Gram-positive.

Spore formation. When first isolated, spores were formed early, often in 24 to 48 hours. After long cultivation in the laboratory, they appear in the cultures only after 6 to 8 days growth. They are formed in the centers or towards one end of the rods and are

typical mesentericus spores.   They retain definite rims of proto-
plasm at times concentrated at one end of the spore and measure
0.375 to 0.5 by 1 to 1.25 microns.   As they lose their rims of
protoplasm they become more oval and measure about 0.75 by
1 micron.

Agar slant.   Growth scanty in 24 hours, then becomes slightly
raised, finely wrinkled, translucent, non-spreading and viscous.
When older the growth has a tendency to become somewhat
dry and gray and is easily scraped off.   On highly acid agar the
growth is more profuse within 24 hours but is not viscous.

Agar stab.   Slight granular growth along the line of inocula-
tion with occasionally a slight budding out from the stab.

Agar colonies.   Colonies small, drop-like, slightly irregular,
showing little or no tendency to spread, glistening, elevated and
viscous.   In some cases a scum-like covering which enclosed
clear gelatinous material was produced about each colony.

Glucose litmus agar slant.   Moderate non-spreading growth
within 24 hours.   The surface of the growth has a sort of honey-
combed appearance caused by fine interlacing wrinkles.   It is
also viscous and is somewhat bluish-gray in color.   The reac-
tion is definitely acid within 24 hours.   This acidity is followed
by a reduction of the litmus and a gradual return to alkalinity.

Glucose litmus agar colonies.   The colonies correspond closely
to those on plain agar but usually attain somewhat greater
dimensions.

Gelatin stab.   Rapid funnel-like and progressive liquefaction.
Complete liquefaction results with the formation of a tenacious
grayish scum.

Gelatin colonies.   The colonies on gelatin plates rest in cup-
like excavations caused by rapid liquefaction.   A definite brown
center with a surrounding grayish granular area is evident in
each colony under the low power of the microscope.

Broth.   A slight turbidity is produced within 24 hours with
the beginning formation of a scum.   The scum later is finely
granular and is formed of discrete colonies.   The medium clears
itself by sedimentation.

Peptone.   Reaction the same as that in broth.

Potato. Growth finely wrinkled, grayish and viscous. The wrinkles appear wave-like. When older the growth loses its viscosity and becomes dry and granular.

Litmus milk. A gradual clearing from the top due to progressive peptonization occurs within 24 hours. Within 48 hours peptonization is generally completed. The remaining fluid has a port-wine color but becomes amber-colored after a variable period.

Blood serum. Smooth, moist, glistening and viscous growth. Within 24 hours some solution of the medium occurs along the line of inoculation (a trough-like excavation). The medium may be entirely dissolved within 2 weeks, and a tenacious scum may be formed.

Fermentation tubes. Glucose: finely granular scum is formed which generally breaks up into large flakes. A flocculent growth is present in the bowl. The reaction is definitely acid.

Saccharose: the growth is the same and the reaction also acid.

Lactose: the growth is also abundant with more pronounced scum-formation. Reaction alkaline.

Thermal death point. The spores survive 10 pounds in the autoclave but are destroyed by 15 pounds pressure. They survive 30 minutes steaming in the Arnold but are destroyed by one hours exposure.

BACILLUS ADHAERENS nov. sp.

This organism has been encountered but once, in dust. It is apparently a new species.

Morphology. Slender long rods with homogeneous protoplasm and flat ends, growing usually in long curved chains made up of 18 to 20 elements. In young cultures on plain agar the individual cells measure 0.375 to 0.5 by 1.5 to 4 microns. Some longer forms, 6 microns in length, may also be found. On glucose agar the organisms are homogeneous, measure 0.625 to 0.75 by 3 to 5 microns, but are often longer measuring 6 to 8 microns. In older cultures (4 days) many globular bodies occur on glucose agar. They resemble the globular bodies seen in *Bacillus cereus.* (Figures 63 and 64.)

Motility. No motility has ever been observed, even in very young cultures.

Staining properties. Gram-positive.

Spore formation. When first isolated this species formed spores in 24 hours. After long artificial cultivation spores are formed only after 4 to 5 days growth on both plain and glucose agar. They are usually subterminal but may be central. The rods swell appreciably before sporulation, sometimes in the center and sometimes at the ends. The free spores are oval and measure 0.625 to 0.75 by 0.875 to 1 micron. They often remain fastened to each other in long chains. Frequently a bit of protoplasm remains attached to the spore which then resembles a tennis racket with its handle.

Agar slant. In early growth (18 hours) this species slightly resembles *Bacillus mycoides.* The line of inoculation shows a distinct ridge from which shoot out fine interlacing filaments. These adhere closely to and grow into the agar. Considerable puckering of these interlacing filaments causes a roughened leathery appearance on the surface. The early growth is moist and slightly glistening but these properties are soon lost. The edges of the growth are serrated, with little or no tendency to spread. A brownish color is found in old cultures throughout the entire medium.

Agar slant. Profuse growth along the line of inoculation and out into the medium. This has the appearance of an inverted fir tree.

Agar colonies. The colony is very characteristic. It first appears like a small colony of *Bacillus mycoides,* but within 24 hours the filaments seem to swell and produce a somewhat corrugated surface with a very definite, elevated and yellow-brown center. The entire colony adheres closely to the agar and gradually grows into it.

Glucose litmus agar slant. The growth is similar to that on plain agar but is very scanty. A definite and permanent acidity is produced.

Glucose litmus agar colonies. Colonies in this medium are considerably smaller than those on plain agar and have the same general appearance.

Gelatin stab. In gelatin, growth is slow and a very slow funnel-like liquefaction is produced.

Gelatin colonies. They appear coarsely granular, slightly raised with definite yellow-brown centers. The centers are coarsely flocculent under the low power of the microscope. The colonies are surrounded by a slight area of liquefaction.

Broth. A slight turbidity is produced and a definite scum is formed which settles to the bottom.

Peptone. Growth similar to that in broth.

Potato. A fairly profuse grayish-white moist growth. When the medium is dry the growth is scale-like.

Litmus milk. No change is noticed within 24 hours. After 48 hours a slowly progressive peptonization occurs. The medium becomes amber-colored.

Blood serum. A fine-grained leather-like growth occurs, dull gray and adherent to the medium. Later this is easily scraped off. No solution of the serum occurs.

Fermentation tubes. Glucose: a flocculent growth occurs in the bowl and extends into the closed arm. Reaction acid.

Saccharose: turbidity in bowl. Arm clear. Reaction neutral or slightly acid.

Lactose: turbidity in bowl. Arm clear. Reaction not changed.

Thermal death point. The spores resist 18 pounds pressure in the autoclave but are destroyed by 20 pounds. They survive one hour's steaming in the Arnold.

SPORE-BEARING ORGANISMS IN WATER

BY C. A. LAUBACH

The spore-bearing organisms in water were obtained by passing the tap water in the laboratory through Berkefeld filters under pressure for a period of three days, washing the filters in sterile salt solution, heating the washings to 80°C. for 15 minutes and then plating. Ten samples were obtained by this method and 313 organisms studied. The species previously established from studies of milk and dust were found as follows:

Bacillus cereus Frankland... 93
Bacillus subtilis (Ehrenberg) Cohn.............................. 84
Bacillus vulgatus (Flügge) Migula................................. 45
(*Bacillus mesentericus vulgatus* Flügge.)
Bacillus mycoides Flügge... 41
Bacillus megatherium De Bary..................................... 14
Bacillus mesentericus (Flügge) Migula........................... 12
(*Bacillus mesentericus fuscus* Flügge).
Bacillus petasites Gottheil......................................   8
Bacillus aterrimus Lehmann & Neumann...........................   7
(*Bacillus mesentericus niger* Lunt.)
Bacillus fusiformis Gottheil.....................................   2
Bacillus brevis Migula...   2
Bacillus prausnitzii Trevisan....................................   1
(*Bacillus ramosus liquefaciens* Prausnitz.)
Bacillus ruminatus Gottheil......................................   1

In addition to the above types one organism was found which had not thus far been isolated in our laboratory but which was described originally by Chester as a viscous variety of *Bacillus subtilis*. Another sporulating organism new to us, producing a green pigment, was found on one occasion. An attempt was made to identify this as one of the numerous fluorescent sporulating organisms already described in water but the morphology and cultural reactions were quite the same as those of *Bacillus cereus* and it was therefore decided to regard it as a fluorescent variety of this species. It has subsequently been isolated on a number of occasions. An organism was encountered on one instance which had such peculiar properties as to merit description as a new species. Therefore the following may be added to the above list.

Bacillus subtilis var *viscosus* Chester........................... 1
Bacillus cereus var *fluorescens* nov. var......................... 1
BACILLUS LATEROSPORUS nov. sp.. 1

Bacillus subtilis variety *viscosus* Chester 1903

Attention was first called to this organism by Chester (1903). We have encountered it but once, in water.

Morphology. Homogeneous rods with round and flat ends measuring 0.5 to 0.625 by 1.5 to 4 microns in young cultures on plain agar. No change in morphology on glucose agar. Trans-

parent or shadow forms abundant on plain and on glucose agar. These measure usually 0.75 to 1.125 by 2.5 to 4 microns. In old cultures an abundance of viscous substance appears staining slightly with gentian violet. (Figures 65, 66 and 67.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. When first isolated, spores were formed within 48 hours. After long cultivation on artificial media, the spore formation has become considerably delayed. Spores are now formed only in ten to fourteen days. They are central or slightly ex-centric and resemble the spores of *Bacillus subtilis.* They measure usually 0.5 to 0.625 by 1 to 1.125 microns and are thus oval to cylindrical. They do not bulge the organisms when formed.

Agar slant. Within 48 hours there is a fair growth which is moist, glistening, non-spreading, glairy and transparent. Its characteristic quality is its viscosity. Later the growth becomes dull and grayish in its appearance and loses its viscosity. A slight wrinkling appears along the outer edge of the growth and forms a narrow border surrounding the entire line of growth. After a few days the medium acquires a brownish color.

Agar stab. A faint granular growth along the line of inoculation.

Agar colonies. Colonies round, definitely circumscribed, with but little tendency to spread, raised, dull and viscous. There is a scum-like covering which encloses gelatinous material about each colony which is very characteristic.

Glucose litmus agar slant. The growth is bluish-gray, viscous, wrinkled and dull. A slight reduction of the litmus occurs with a definite acidity. There is a later return to alkalinity usually within ten days.

Litmus glucose agar colonies. The colonies are similar to those on plain agar but are more profuse and have a definite bluish-gray color.

Gelatin stab. Rapid progressive surface liquefaction. Complete liquefaction occurs within 96 hours with the formation of a definite flocculent sediment.

Gelatin colonies. The colonies are definitely circumscribed and rest in a cup-like excavation due to liquefaction. They are translucent, grayish, viscous with opaque centers.

Broth. A granular, lace-like scum is formed which settles to the bottom. A flocculent growth occurs throughout the medium.

Peptone. Growth similar to that in broth.

Potato. A profuse, grayish, slightly glistening and viscous growth is produced. More characteristic is the appearance of numerous blebs filled with gelatinous material on the entire medium within 24 hours. Later the growth becomes dry and scale-like.

Litmus milk. Within 24 hours a slight reduction of the litmus. Within 24 hours a thickening occurs at the bottom and peptonization begins. This progresses rapidly and within four days the process is generally completed. There remains first a port-wine, then an amber-colored fluid.

Blood serum. The growth is scant, glistening and bluish-gray. There is occasionally a fine wrinkling. A gutter-like excavation along the line of inoculation is often visible. Within 96 hours a pinkish tinge is produced in a wrinkled scum if water of condensation be present at the bottom of the tube.

Fermentation tubes. Glucose: a turbid growth occurs in the bowl and neck with the formation of a thin smooth bluish-gray scum. Some acidity is produced.

Saccharose: a similar growth is apparent but the scum is finely granular. Here also a definite acidity is produced.

Lactose: the growth is identical with that in saccharose but the reaction remains neutral or becomes alkaline.

Thermal death point. Spores survive 15 pounds in the autoclave but are killed by 20 pounds pressure. They are killed within an hour in the Arnold sterilizer.

Bacillus cereus variety *fluorescens*. nov. var.

An organism producing a greenish fluorescence has been encountered frequently in water and in soil. It does not agree with any previously described organisms in its cultural reactions,

but has the same morphology and in many respects the same reactions on media, as *Bacillus cereus*. It has therefore been described as a fluorescent variety of this organism.

Morphology. In young cultures on plain agar (6 to 20 hours) the vegetative rods have rounded ends, slightly granular protoplasm and measure 0.5 to 0.75 by 1.5 to 4 microns. They frequently grow in short chains. On glucose agar they are slightly thicker and longer measuring 0.75 to 0.875 by 2 to 5 microns. As the organisms mature they show many globular bodies in their protoplasm which take the stain with difficulty. These are particularly abundant on glucose agar. (Figures 68, 69, and 70.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form early appearing in 24 hours on plain and on glucose agar. They are located in the center or towards one end of the vegetative rods which swell noticeably on sporulation. As the spores mature the protoplasm disintegrates but remains attached to the spore, sometimes in equal quantities at the ends and sometimes in greater amount at one end than at the other. The spores thus resemble mesentericus spores but are much larger. The free spores measure 0.5 to 0.75 by 0.75 to 1.5 microns. They frequently remain attached to each other in long chains.

Agar slant. The growth is profuse, smooth, moist, and dull. There is a definite ridge along the line of inoculation and the edges are thin and slightly serrated. A yellow-green pigment is diffused throughout the entire medium. This remains permanently and increases with age. The pigment is especially pronounced in an acid medium. Rarely there is considerable wrinkling and the formation of rather coarse folds.

Agar stab. Slight granular growth along the line of stab with the gradual appearance of the characteristic pigment.

Agar colonies. Irregular colonies which have a great tendency to spread in amoeboid fashion. They are glistening, slightly translucent and slightly raised. They become more dense and dull as growth progresses and assume a greenish-yellow coloration. The colonies can be scraped off and are of a

mealy consistency.   The same pigment appears in the medium.

Glucose litmus agar slant.   The growth is fairly profuse and similar to that on agar.   The reaction is first acid with a return to alkalinity after a considerable period.

Glucose litmus agar colonies.   The colonies are similar to those on agar but more profuse.   Reaction of medium acid.

Gelatin stab.   Very rapid liquefaction along the line of inoculation.   The medium is entirely liquefied within 48 hours and acquires an intense yellow-green color.   A scum of the same color is formed.

Gelatin colonies.   The colonies rest in cup-like excavations caused by liquefaction.   They have a definite greenish center surrounded by a net-work of fine interlacing, clear, thread-like filaments.   The medium assumes the characteristic fluorescence.

Broth.   Profuse, flocculent growth with the production of a slight green-yellow coloration.

Peptone.   Growth more pronounced than in broth and the coloration more intense.   A scum is also formed.

Potato.   Profuse, moist, mealy, yellow-green growth with a coloration of the medium.

Litmus milk.   Slight reduction of the litmus with a suggestion of peptonization within 24 hours.   A precipitation of the proteins occurs within 48 hours and peptonization progresses rapidly.   A scum is formed and the fluid remaining is deeply colored with the characteristic pigment.

Blood serum.   Moist, profuse, dull and slightly greenish-yellow growth.   No liquefaction.

Fermentation tubes.   Glucose: profuse flocculent growth with the formation of yellow-green scum in the bowl and a turbid growth in the closed arm.   The usual fluorescence is present.   A definite acidity is produced.

Saccharose: the growth is identical with that in glucose but no acidity is produced.

Lactose: the growth is the same.   Reaction unchanged.

Thermal death point.   The spores survive 12 pounds pressure in the autoclave but are destroyed by 15 pounds.   They are also destroyed by one hour's exposure to steam in the Arnold sterilizer.

BACILLUS LATEROSPORUS nov. sp.

This organism was obtained but once, from water. It is apparently a new species.

Morphology. The typical vegetative rods are found only in very young cultures 6 to 8 hours old. They are homogeneous or slightly granular with round ends, measuring 0.375 to 0.5 by 1.125 to 4 microns on plain agar. On glucose agar they are slightly thicker and about the same length measuring 0.5 to 0.75 by 1.125 to 4 microns. The rods rapidly lose their characteristic appearance and assume a fusiform or spindle shape with a swollen middle and pointed ends. Such forms take the stain much less intensely. (Figures 71 and 72.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. As the organisms swell and assume the fusiform shape the spores appear at one side as slightly staining globules. The protoplasm soon disintegrates but more on one side of the spore than on the other so that the free spores which are almost egg-shaped retain a rim of protoplasm on one side and present a characteristic appearance. Such spores measure 0.75 to 0.875 by 1.5 microns. As the spores mature they lose their rims of protoplasm, become more oval and measure 0.75 to 1 by 1 to 1.125 microns. The spores begin to appear in about 48 hours and are abundant in 72 hours. Rarely small tags of protoplasm remain attached at the ends of the spores.

Agar slant. The growth is very characteristic. It is profuse, rapidly spreading, smooth, moist, and has a silvery metallic-like lustre. Within 72 hours the growth becomes slightly wrinkled. With age the growth loses its metallic lustre and becomes dull and scrapes off in scales.

Agar stab. A slight grayish granular growth along the line of stab.

Agar colonies. The colonies are flat, transparent, irregular, moist, and have the same peculiar metallic lustre.

Glucose litmus agar slant. Growth slight, granular, moist and gray. Slight reduction of the litmus within 24 hours and a permanent acidity within 96 hours.

Glucose litmus agar colonies. Colony formation is very slow and the colonies only appear within 48 hours. They are similar to those on agar but less profuse. At times a slight wrinkling occurs.

Gelatin stab   Very slow surface liquefaction.

Gelatin colonies. The colonies are definitely circumscribed, gray and moist, under the low power of the microscope showing finely granular centers surrounded by small gray rings. Beyond the rings there are other granular areas the outer borders of which consist of dense gray rings. A very peculiar odor like that of semen plus some aromatic radical occurs in gelatin.

Broth. Peculiar pellicle made up of discrete colonies which sink to the bottom. There is also a slight turbidity.

Peptone. Growth similar to that in broth.

Potato. The growth is dull, moist, gray, granular and finely wrinkled. A slight lustre appears which becomes more pronounced as the medium becomes drier.

Litmus milk. Within 24 hours there is a complete reduction of the litmus with an alkaline ring at the surface. A soft coagulum is formed within 24 hours which is somewhat firmer than the coagulum formed by other spore-bearing organisms. Peptonization begins after 48 hours and requires a considerable period before it is completed. The fluid remaining is straw-colored.

Blood serum. The growth is grayish, moist, non-spreading and not profuse. Within 96 hours it becomes finely wrinkled.

Fermentation tubes. Glucose: there is a heavy pellicle formed and a turbid growth in the bowl and neck of tube. If litmus is present it is reduced in the closed arm. Also a turbid growth is found in this arm. Reaction acid.

Saccharose: a similar condition is apparent with little or no acidity.

Lactose: there is the same reaction with a slight alkalinity.

Thermal death point. The spores survive 12 pounds pressure in the autoclave but are destroyed by 15 pounds pressure. They are destroyed by one hour's steaming in the Arnold sterilizer.

SPORE-BEARING BACTERIA IN SOIL

BY C. A. LAUBACH AND J. L. RICE

In a preliminary investigation of the germ content of soils obtained in the neighborhood of Baltimore the attempt was made to verify the results already obtained by the work on milk, dust and water and to determine whether the types previously isolated from the other sources could also be found in this environment. Altogether some 63 cultures were obtained and found to consist of the following types:

Baltimore soil

Bacillus cereus Frankland.. 28
Bacillus mesentericus (Flügge) Migula.............................. 15
(*Bacillus mesentericus fuscus* Flügge.)
Bacillus subtilis (Ehrenberg) Cohn................................. 8
Bacillus vulgatus (Flügge) Trevisan 6
(*Bacillus mesentericus vulgatus* Flügge.)
Bacillus brevis Migula.. 3
Bacillus megatherium De Bary..................................... 1
Bacillus mycoides Flügge... 1
Bacillus petasites Gottheil...................................... 1

It is interesting to note the great predominance of *Bacillus cereus* which makes up nearly half the isolations, the relative infrequency of *Bacillus subtilis* and the rarity of *Bacillus megatherium, Bacillus petasites,* and *Bacillus mycoides.* `Bacillus mesentericus* appears also as a more frequent isolation than *Bacillus vulgatus.*

Eight different samples of raw and heated soil were selected for this examination, some samples being plated immediately, others being incubated at 37°C. for 48 hours before plating. It was hoped that a great diversity of organisms would be obtained by this method and no special attempt was made to estimate with any great accuracy the germ content of any particular sample. In general it was noted however, that heated soil furnished a greater variety of types than unheated soil and that the incubation of the sample was distinctly an unfavorable factor since one type tended to overgrow the other types present. It was thought that the prevalence of *Bacillus cereus* might be due to its power of

crowding out the other microörganisms with which it was associated in soil, rather than to its original presence in greater numbers.

In a subsequent investigation 520 cultures were obtained from 8 soils, 5 of them from the vicinity of Baltimore and 3 from Nazareth, Pennsylvania. The heating of the soil to temperatures of 60° and 80°C., and the incubation of the sample was abandoned owing to the inconsistency of the results obtained and the overgrowth by *Bacillus cereus*. All samples were boiled for 20 minutes and plated immediately. Of the 520 cultures 306 were from Baltimore and 214 from Nazareth. The cultures included the following previously described types:

	Baltimore Soil	Nazereth Soil
B. petasites Gottheil	73	116
B. cereus Frankland	134	41
B. megatherium De Bary	29	13
B. subtilis (Ehrenberg) Cohn	24	9
B. mesentericus (Flügge) Migula	9	11
(Bacillus mesentericus fuscus Flügge.)		
B. vulgatus (Flügge) Trevisan	12	6
(Bacillus mesentericus vulgatus Flügge.)		
B. mycoides Flügge	15	2
B. mesentericus var. flavus		9
B. cereus var. fluorescens	3	
B. fusiformis Gottheil	3	2
B. brevis		3
B. simplex Gottheil	1	
B. cohaerens Gottheil	1	2
	304	214

One new species (BACILLUS AGRI) belonging to the Cohaerens-simplex group was isolated from the Baltimore soil on two occasions.

In the results obtained from Baltimore it is interesting to note the predominance of *Bacillus cereus*, the large number of isolations of members of the Megatherium-petasites group and the small number of *Bacillus mycoides*. *Bacillus mycoides* indeed is a rare organism in Baltimore and soil conditions here apparently differ markedly from those in other localities. *Bacillus subtilis* and the members of the mesentericus group are also uncommon

while the other organisms mentioned are extremely rare.   The conclusions reached by the study of the total number of organisms are borne out by the analysis of the individual samples which may be tabulated as follows:

TYPE	BALTIMORE SERIES					NAZARETH SERIES		
	1	2	3	4	5	6	7	8
B. cereus..................	16	20	23	17	58	8	10	23
B. petasites...............	10	18	15	21	9	20	30	66
B. mycoides...............	5	6	3	1				2
B. megatherium............	4	6	8	8	3	3	4	6
B. subtilis.................	2	4	3	7	8	1	4	4
B. vulgatus................	1	2	4	4	1	2	2	2
B. mesentericus............			2	6	1		8	3
B. cohaerens..............				1		1		1
B. fusiformis..............					3			2
B. agri....................				1	1			
B. simplex................		1						
B. cereus var. fluorescens..	1		2					
B. mesentericus var. flavus.							7	2
B. brevis..................							2	1
Total..................	39	57	60	66	84	35	67	112

Source of samples: 1, Paving sand, Baltimore City; 2, surface soil, lawn of Johns Hopkins Hospital; 3, soil from fertilized flower bed, Baltimore City, under cultivation, 20 years; 4, soil from cultivated garden, Baltimore; 5, soil from field, suburb of Baltimore; 6, clay from depth of 5 feet, Nazareth; 7, soil from highly fertilized garden, under cultivation more than 25 years; 8, richly fertilized field soil.

In the Nazareth series the predominant organism was *Bacillus petasites* which made up more than half the isolations.   *Bacillus cereus* stood next while *Bacillus mycoides* was obtained on but two occasions.   The analysis of the individual samples shows the same predominance of *Bacillus petasites* and is chiefly interesting in demonstrating the small number of organisms isolated from soil collected at the depth of five feet as contrasted with the more diversified flora of highly fertilized top garden and field soil.

In general it may be seen that the study of soil confirms the conclusions reached by the study of milk, dust and water.   The

predominating types isolated from these latter sources were also found predominating in soil, since the 520 cultures revealed only one new species.

BACILLUS AGRI nov. sp.

This organism was isolated on two occasions from Baltimore soil and cannot be identified with any previously-described species. It evidently belongs in the Simplex-cohaerens group and because of its different cultural reactions must be described as a new species.

Morphology. Large uniform homogeneous rods with round ends measuring 0.625 to 0.75 by 2.5 to 5 microns in young cultures on plain agar. Occasionally chains with a number of elements are found and shadow or transparent forms measuring 12–15 microns in length. On glucose agar there is no special change in morphology, but the proportion of shadow forms is greater. The morphology resembles that of *Bacillus simplex.* (Figures 73 and 74.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form within 24 hours but are not abundant till after the lapse of three or four days. They are central or slightly ex-centric, no wider than the rods from which they spring. The free spores retain rims of protoplasm for some time. They measure 0.625 to 1.125 to 1.25 microns. As they lose their protoplasm they become more oval and measure 0.5 to 0.625 by 0.75 to 1.125 microns. The spore-formation resembles that of *Bacillus simplex.*

Agar slant. Fairly profuse gray moist smooth glistening opaque growth along the line of inoculation with a slight tendency to spread.

Agar stab. Faint granular growth along line of inoculation.

Agar colonies. Small gray moist glistening elevated colonies with opaque centers. No tendency to spread.

Glucose litmus agar. Fairly profuse gray moist smooth glistening opaque growth with a slight tendency to spread. The litmus is completely reduced within 48 hours with a very slight

initial acidity which is rapidly replaced by a permanent alkalinity. The growth wrinkles slightly.

Glucose litmus agar colonies. Grayish, moist, opaque glistening and slightly wrinkled colonies. The reaction is the same as that on the glucose litmus agar slant.

Gelatin stab. A definite granular growth along the line of inoculation followed by complete liquefaction. The liquefaction is funnel-like.

Gelatin colonies. Definitely circumscribed. grayish, moist, glistening colonies with opaque centers and thin lace-like peripheries. The colonies rest in excavations caused by liquefaction. On examination with the low power, they appear to be composed of large flocculi.

Broth. A very turbid growth which clears by sedimentation. No pellicle is formed.

Peptone. Growth similar to that in broth.

Potato. Fairly abundant, moist, heaped up, glistening, viscous and grayish growth.

Litmus milk. No change is noticed within 96 hours, after which period a slight reduction becomes evident. The reduction is completed within about 7 days and then the milk is slowly peptonized. Peptonization is generally completed within 14 days. The remaining liquid is at first grayish in color but after a variable period becomes amber-colored.

Blood-serum. A fairly abundant, thin, smooth, slowly spreading, slightly glistening, brownish growth occurs. No liquefaction of the medium.

Fermentation tubes. Glucose: a turbid growth occurs in the bowl and neck but does not extend up the closed arm. No scum is formed and a very slight initial acidity is produced which gives way to a slight alkalinity.

Saccharose: the growth corresponds closely to that in glucose. There is a slight alkalinity.

Lactose: the growth is similar with the production of a slight alkalinity.

Thermal death point. The spores survive 10 pounds in the auto-

clave but are destroyed by 12 pounds pressure. In the Arnold they survive 30 minutes steaming but are destroyed by one hour's exposure.

MISCELLANEOUS CULTURES

BY WILLIAM W. FORD

On a number of occasions miscellaneous cultures from a variety of sources have been studied with care and identified. Such cultures, about 100 in number, include spore-bearing organisms found at times as laboratory contaminations, in the normal and abnormal dejecta of children and adults, in milk products, etc. The majority of such cultures were easily identified and found to correspond to the well-known types already described. The most frequent isolation was *Bacillus cereus* which is our common laboratory contamination. Next to this organism *Bacillus subtilis* was most frequent, then *Bacillus vulgatus* or *Bacillus mesentericus*. Members of the Megatherium-petasites group were also not uncommon. In addition to such isolations as these *Bacillus circulans* of Jordan was found on one occasion. It is recognized by its cylindrical terminal spores and by its cultural reactions. Dr. Jordan was kind enough to examine the culture and confirmed our identification. *Bacillus circulans* is evidently one of the rarer sporulating bacteria. At another time a round-spored organism resembling *Bacillus tetani* in morphology was encountered as a contamination on an old agar plate. This species was apparently first described by Kruse (Flügge, 1896) as an aerobic variety of the tetanus bacillus. On three occasions an organism was isolated which was originally obtained by Flügge (1894) and named later *Bacillus brevis* by Migula (1900). The same species was found by us in Montreal. Finally on one instance a culture was found in the normal dejecta which could not be identified with any previously described organisms. The morphology and spore formation were so characteristic and so different from that seen with other spore-bearing bacteria that it was decided to create a new species for which the name BACILLUS CENTROSPORUS is selected. A detailed description of these organisms follows.

Bacillus circulans Jordan 1890

This organism was described originally by Jordan in 1890. The present culture was obtained from normal human dejecta and corresponds closely with Jordan's description.

Morphology. Rather long and thin bacilli with rounded or square ends measuring about 0.5 by 2.5 to 4 microns in 24 hour agar cultures. Occurs usually as single cells. Protoplasm homogeneous. On glucose agar the organisms are thicker and longer measuring 0.75 by 5 to 8 microns. (Figure 75.)

Motility, Active progressive and rotatory motility in 24 hour agar cultures.

Staining properties. Gram-positive.

Spore formation. Spores form early on plain agar often in 24 hours. Abundant in 48 hours. Form more slowly on glucose agar. They are cylindrical, wider than the rods from which they spring and terminal in position. The free spores may retain rims of protoplasm at one end or may be naked. They measure usually about 0.75 by 1.125 microns.

Agar slant. Thin slightly moist non-spreading growth, pale white in color.

Agar colonies. Round regular non-spreading colonies, under low power pale yellow, finely granular, with entire edges. Older colonies become irregular and amoeboid showing under low power central nuclei and thin peripheries.

Agar stab. Faint growth along line of inoculation spreading at point of puncture.

Glucose agar. Faint thin non-spreading growth producing an acid reaction beginning in 24 hours and quite marked at the end of 5 to 6 days.

Glucose agar colonies. Small fine colonies, under low power pale yellow, granular with entire or serrated edges. Reaction acid.

Gelatin stab. Faint growth along line of inoculation. Little or no surface growth. No liquefaction.

Gelatin colonies. Small fine colonies with hazy outlines, under low power dark opaque with fuzzy margins. Older colonies quite amoeboid. No liquefaction.

Broth. Slight turbidity, no scum, no sediment.

Peptone. Slight turbidity, no scum, no sediment.

Potato. No visible growth.

Milk. Complete decolorization within 24 to 48 hours. Gradual production of acidity and late coagulation, which may appear spontaneously and always occurs if the milk be heated.

Blood serum. Faint thin white non-spreading growth. No liquefaction.

Fermentation tubes. Glucose: turbidity in bowl and closed arm. No scum. Reaction acid, appearing in 3 to 4 days.

Saccharose: appearance the same. Reaction acid.

Lactose: appearance the same. Reaction acid.

Thermal death point. Spores destroyed by steaming 15 minutes in the Arnold. Survive 18 pounds pressure in the autoclave. Destroyed by 20 pounds pressure.

Bacillus pseudotetanicus (Kruse) Migula 1900

This organism was first described by Kruse in the 3rd edition of Flügge's "Die Mikroorganismen" under the name *Bacillus pseudotetanicus* var. *aerobius*. It resembled *Bacillus tetani* in morphology and spore-formation but was distinguished from it by its aerobic development, its failure to liquefy and its lack of pathogenicity. Apparently the same organism has been described by Neide (1904) as *Bacillus sphaericus*. The species here described has the morphological and cultural features given by both Kruse and Neide and under the rules of nomenclature the name given by Migula should be adopted. It was found but once as a contamination on an agar plate.

Morphology. Short thick bacilli with rounded ends occurring as single cells or as two cells end to end. In 24 agar cultures they measure 0.75 by 1.5 to 3 microns. Protoplasm homogeneous. Many of the cells are fusiform, distinctly swollen toward the middle while others are swollen at the ends. On glucose agar the organisms are longer and thicker measuring 1.125 by 1.5 to 4 microns. They show no change in the character of the protoplasm. In older cultures the organisms often form long threads measuring 8 to 15 microns in length. (Figures 76 and 77.)

Motility. Active and progressive rotatory motility in 24 hour agar cultures.

Staining properties. Gram-negative.

Spore formation. Spores are formed early often within 24 hours on both plain and glucose agar. They are round, and usually appear at the ends of the organisms in a sub-terminal position, but may be distinctly terminal. The spores are of greater diameter than the vegetative rods from which they spring and thus give a clavate or club-shaped appearance to the organisms. The organisms taken from glucose agar resemble markedly the tetanus bacillus cultivated on this medium. The free spores retain fairly thick walls of protoplasm for some time. They vary in size from 1 to 1.125 microns in diameter.

Agar slant. Moist rather thin translucent growth in 24 hours, becoming pale yellowish white in old cultures. No tendency to spread except when the medium is very moist.

Agar stab. Faint growth along line of inoculation with slight spreading on the surface. In older cultures the surface growth is thicker and more abundant but does not usually spread far from the line of puncture. Easily scraped from medium.

Agar colonies. Colonies round and regular or amoeboid in 24 hours. Under low power they show dark central areas with lighter peripheries. As they age they become thicker and denser with more pronounced central nuclei.

Glucose agar. Pale white moist rather thin growth in 24 hours, becoming pale yellow or cream yellow in older cultures.

Glucose agar colonies. In 24 hour plates the colonies are irregular, amoeboid, thick, showing under low power darker central nuclei with lighter peripheries but with entire edges. As the colonies become older they become denser and thicker with heaped-up edges. They give a peculiar bizarre appearance to the plate as though it was covered with drops of moisture. Reaction of agar remains neutral or becomes alkaline.

Gelatin stab. Faint line growth appearing after 48 to 72 hours with a slightly spreading growth on the surface. In older cultures the surface growth spreads considerably from the point of puncture on top of the gelatin which remains solid. Rarely the line growth becomes arborescent.

Gelatin colonies. Small fine colonies visible only after 48 hours. Under low power they are round, regular and pale yellow with entire margins. As they grow older they become denser and more opaque and under low power show central nuclei with pale margins. Colonies two weeks old are thick brownish non-spreading. No liquefaction.

Broth. Turbidity and rather faint sediment. No scum.

Peptone. Turbidity with slight sediment. No scum.

Potato. Rather scanty yellowish moist growth becoming brown in old cultures.

Litmus milk. No change in appearance of the milk in the first few days then a gradual reduction of the litmus, completed in 15 to 18 days. No peptonization even in milk cultures kept under observation four weeks. No coagulation.

Blood serum. Dry thin yellowish growth becoming pale yellow-brown in old cultures. No liquefaction.

Fermentation tubes. Glucose: turbidity in bowl, no scum, arm clear. Reaction alkaline.

Saccharose: reaction the same.

Lactose: reaction the same.

Thermal death point. The spores resist steaming in the Arnold sterilizer 15 minutes but are destroyed in 30 minutes. They resist a pressure of 14 pounds in the autoclave but are destroyed by 16 pounds.

Bacillus brevis Migula 1900

This organism was first described by Flügge (1894) as BACILLUS No. I. According to Neide (1904) who has given an accurate account of its morphology and cultural reactions under the name *Bacillus lactis* Flügge, it is probably identical with *Bacillus cylindrosporus* Burchard (1898). The correct name is that of Migula. Three different strains have been isolated from milk, from soil, and from dust. They agree in their cultural reactions with the description given by one of us (W. W. F.) of a culture isolated in Montreal.

Morphology. Bacilli with pointed ends and slightly granular protoplasm, sometimes fusiform in shape. In 24 hour cultures on plain

agar they measure 0.375 to 0.5 by 2 to 3 microns. On glucose agar they are more uniform and slightly thicker and longer, measuring usually 0.5 by 3 to 4 microns. (Figures 78, 79 and 80.)

Motility. Active progressive motility in 24 hour agar cultures.

Staining properties. Gram-negative.

Spore formation. Spores are formed early appearing often within 24 hours on plain and glucose agar. They are cylindrical, usually subterminal in position, thicker than the organisms from which they spring, and present a characteristic appearance. They may retain spurs of protoplasm at each end or may be entirely naked. They measure usually 0.75 by 1.125 to 1.5 microns. Rarely they may be distinctly terminal in position.

Agar slant. Thick moist spreading growth with a silvery sheen abundant in 24 hours and becoming thick white and glistening in older cultures. Easily scraped from medium.

Agar stab. Filiform growth along line of inoculation with an abundant surface growth spreading to the tube wall.

Agar colonies. Surface colonies in 24 hours are round and regular or thin spreading translucent, under low power slightly granular with entire edges. Deep colonies small fine, under low power irregular and pale yellow. As the superficial colonies get older they become thicker and heaped up slightly. The deep colonies may show irregular fuzzy margins under the low power.

Glucose agar. Heaped up white moist non-spreading growth in 24 hours, becoming thick and raised in old cultures. Reaction alkaline.

Glucose agar colonies. Superficial colonies are slightly thicker than plain agar colonies in 24 hours and show a greater tendency to spread. Under the low power they are granular with entire edges. Deep colonies small and fine, under low power irregular and pale yellow. Older superficial colonies are thick, irregular and heaped up. Reaction of agar alkaline.

Gelatin stab. Faint growth along line of inoculation and at the point of puncture in 3 to 4 days, with beginning liquefaction. Distinct cup-shaped or funnel liquefaction in two weeks. Occasionally a fragile scum appears on the liquid gelatin.

Gelatin colonies. Colonies begin to appear in 48 hours and show

dark central areas with hazy peripheries. Under the low power they show dark opaque centers with wavy branching peripheries. Deep colonies frequently show peculiar prickly margins. Gelatin completely liquefied at the end of about two weeks and may show a thick scum on the surface.

Broth. Turbidity and a flocculent sediment in 24 to 48 hours with a gradual production of a heavy scum which appears often only after 4 days. In old cultures the precipitate is quite heavy.

Peptone. Turbidity and heavy flocculent sediment. Scum may be thick and heavy or entirely lacking.

Potato. Usually a rather scanty moist yellow or yellow-brown growth.

Milk. No change in reaction. Litmus begins to decolorize in 48 hours and by the end of two weeks is completely decolorized. At the same time the proteins are digested and the resulting fluid is a pale rather thin colorless solution. No coagulation.

Blood serum. Thin cream-white non-spreading growth becoming yellowish-brown in old cultures. No liquefaction.

Fermentation tubes. Glucose: thick scum, turbidity in bowl, arm clear. Reaction alkaline.

Saccharose: reactions the same.

Lactose: reactions the same.

Thermal death point. The spores survive steaming 30 minutes but are destroyed by 45 minutes exposure. They may survive a pressure of 16 pounds in the autoclave but are destroyed by 18 pounds pressure.

BACILLUS CENTROSPORUS nov. sp.

This is a new species obtained but once, from the normal dejecta of a child from the Harriet Lane Home.

Morphology. In 24 hour cultures on plain agar at 37°C. or in 36 hour cultures at 22°C. long thin bacilli with pointed ends fusiform or clostridium in shape with slightly granular protoplasm (grassy), occurring usually as single cells measuring 0.375 to 0.5 by 3 to 4.5 microns in dimensions. The majority of the

organisms measure about 3 to 4 microns in length. On glucose agar the organisms measure about 0.375 by 2.25 to 3 microns and the ends are rounder but there is no change in the character of the cytoplasm. (Figures 81 and 82.)

Motility. Active progressive and rotatory motility in 24 hour agar culture.

Staining properties. Organisms stain readily by the ordinary dyes. Gram-negative.

Spore formation. Spores are formed early appearing in 24 hours on plain agar in the thermostat, somewhat later on glucose agar. They are usually formed in the central portion of the rods causing a distinct swelling so that the bacilli become spindle-shaped. Rarely the spores appear toward the end of the rods in a subterminal position or they may even be terminal. They are much wider than the vegetative rods and when free are distinctly cylindrical with pointed spurs. They measure 0.625 by 1.125 to 1.5 microns. By the end of 48 hours both at 37° and at 22° the spores are abundant.

Agar slant. Thick moist rather dark non-spreading growth in 24 hours, becoming thicker and raised in 48 hours and in older cultures. Easily scraped off from medium.

Agar stab. Growth along line of inoculation in 24 hours, gradually spreading on the surface of the agar at the point of puncture.

Agar colonies. Round regular rather translucent colonies in 24 hours, granular with smooth margins under the low power. Older colonies show pale central areas with heaped up peripheries but remain small regular and slightly raised, resembling those of *Bacillus coli.*

Glucose agar slant. Thick moist cream-yellow non-spreading growth in 24 hours, becoming thick heavy with an irregular surface of a reddish brown color in old cultures. Reaction alkaline.

Glucose agar colonies. Irregular heaped up colonies in 24 hours tending to become slimy or viscid-looking. Under low power they are irregular, heaped up or swollen, with entire margins. At times they have a dew-drop appearance. On old cul-

tures (10 days) they are thicker and slightly brownish.   Reaction of medium alkaline.

Gelatin stab.   Filiform growth along line of inoculation and gradual liquefaction in the form of a sphere below the surface. Liquefaction is slow, taking place only after 7 to 8 days.   Liquefaction gradually reaches the walls of the tube.   Growth not arborescent.

Gelatin colonies.   Colonies appear only after 5 to 6 days. They are small fine and surrounded by a hazy zone.   Gelatin slowly liquefied.   Under low power colonies are round regular finely granular with entire margins or may show irregular outlying strands.

Broth.   Turbidity within 24 to 48 hours with a faint friable scum appearing in about 10 days.

Peptone.   Turbidity within 24 to 48 hours with faint friable scum in 10 days.

Potato.   Moist yellow growth within 24 hours gradually becoming heaped up and irregular and assuming a brown or reddish brown color.

Litmus milk.   No change in reaction except for an occasional partial reduction of the litmus.   No peptonization.   No coagulation.

Blood serum.   Pale yellow growth in 24 hours gradually becoming thick and moist in old cultures.   No liquefaction.

Fermentation tubes.   Glucose: turbidity in bowl, no scum, arm clear.   Reaction alkaline.

Saccharose: reactions the same.

Lactose: reactions the same.

Thermal death point.   The spores stand boiling 15 minutes but are destroyed by boiling for 30 minutes.   They resist 15 minutes' exposure in the Arnold but are destroyed by 30 minutes' exposure. They resist autoclaving at a pressure of $12\frac{1}{2}$ pounds but are destroyed by 14 pounds pressure.

CLASSIFICATION

BY W. W. FORD

From a study of spore-bearing organisms lasting over a period of four years, during which over 1700 cultures were worked out from a variety of sources including milk, dust, water, soil, intestinal contents, and laboratory stock cultures 28 species or varieties of species were isolated and established as distinct types by the usual morphological, developmental, tinctorial and cultural features available for systematic investigation. The majority of these types were encountered on many separate occasions and our descriptions of them are based upon those fixed and permanent characters which we regard as establishing bacterial species. These types thus represent the organisms met with in our work quite regardless of their identification as previously established species or varieties. Of the total number, 28, we were able to identify 22 as species already described in the literature. Whether these species are correctly named, is a matter about which of course opinions may vary. We feel however that our identification of these 22 species is as satisfactory as the confusion in the literature and the uncertainty of stock cultures permit. In addition to the 22 types described by previous workers two distinct varieties of old species were found while four organisms were isolated which are so different from any described in the literature as to merit establishment as new species. To these six organisms new names have been given. With these 28 types in mind it now becomes possible to classify the spore-bearing organisms from a preliminary standpoint. The basis for the classification rests primarily upon morphology, spore-formation and motility, and secondarily upon tinctorial and cultural features. All the characters are easily demonstrated in any properly equipped bacteriological laboratory. Both the microchemical properties of the bacterial cells and the methods of spore-germination while studied when possible have been disregarded in arranging the species in groups. The extent to which this classification is of permanent value will be shown, we believe, by the approval or disapproval with which it is received by American bacteriologists.

AEROBIC SPORE-BEARING NON-PATHOGENIC BACTERIA

Group I.   Subtilis group

Small homogeneous sluggishly motile organisms measuring 0.375 by 1.5 to 2.5 microns.   No threads on glucose agar.   Central or ex-centric spores, oval, measuring 0.5 by 0.75 to 0.875 microns, often retaining terminal tags of protoplasm.   Growth on solid media hard and penetrating, with tenacious scums on fluid media.

Represented by
   Bacillus subtilis Cohn.
   Bacillus subtilis-viscosus Chester.
      (Characterized by viscosity.)

Group II.   Mesentericus group

Small homogeneous actively-motile organisms measuring 0.5 by 2 to 4 microns.   Often produce long threads on glucose agar. Spores measure 0.5 by 1 to 1.125 microns, oval, retaining terminal tags of protoplasm.   Growth on hard media as soft pultaceous mass with tendency to wrinkle, on fluid media as friable easily-broken scums.

Represented by
   Bacillus vulgatus (Flügge) Trevisan.
      (*Bacillus mesentericus vulgatus* Flügge.)
   Bacillus mesentericus (Flügge) Migula.
      (*Bacillus mesentericus fuscus* Flügge.)
   Bacillus aterrimus Lehmann & Neumann.
      (*Bacillus mesentericus niger* Lunt.)
   Bacillus globigii Migula.
      (*Bacillus mesentericus ruber* Globig.)
   Bacillus niger Migula.
      (*Bacillus lactis niger* Gorini.)
   Bacillus mesentericus var. *flavus*.
   Bacillus panis Migula.
      (*Bacillus mesentericus panis viscosi I* Vogel.)
      (Motility lost by capsule formation.)

Group III. Cohaerens-simplex group

Motile organisms somewhat larger than either *Bacillus subtilis* or *Bacillus mesentericus*, measuring 0.375 to 0.75 by 0.75 to 3 microns. Thicker and longer forms on glucose agar. Involution and shadow forms common and appear early. Spores cylindrical, measuring 0.5625 to 0.75 by 1 to 1.5 microns. Growth as a soft mass on hard media, as turbidity with little or no scum on fluids.

Represented by

Bacillus cohaerens Gottheil.

Bacillus simplex Gottheil.

BACILLUS AGRI nov. sp.

Group IV. Mycoides group

Large organisms with square ends growing in long chains. Single cells measure 0.5 by 3 to 6 microns. On glucose agar organisms are thicker and longer and made up of globular bodies. Tendency for organisms to grow in curves or spirals. Spores central or ex-centric, round or oval to cylindrical, measuring 0.75 to 1 by 1 to 2 microns. Spores vary greatly in size and often appear in chains. Growth on hard media dry and penetrating, on fluid media as firm tenacious scum.

Represented by

Bacillus mycoides Flügge.

Bacillus prausnitzii Trevisan.

  (*Bacillus ramosus liquefaciens* Prausnitz.)

BACILLUS ADHAERENS nov. sp.

  (No motility.)

Group V. Cereus group

Large motile organisms with round ends measuring 0.75 by 2.25 to 4 microns. Tend to grow in short chains. Thicker and longer on glucose agar where protoplasm is converted into globular bodies. Central or excentric spores, cylindrical, measuring 0.5 to 0.75 by 1.125 to 1.5 microns. Spores retain proto-

plasm at one or both ends often resembling enlarged subtilis or mesentericus spores.   Growth on hard media as soft pultaceous mass with tendency to fold or wrinkle, on fluid media as thick friable scum.

Represented by

Bacillus cereus Frankland.
Bacillus albolactus Migula.
Bacillus cereus var. fluorescens nov. var.

Group VI.   Megatherium group

Very large actively motile organisms measuring 0.75 to 1.25 by 3 to 9 microns.   Often in long forms which spread out, lose their cytoplasm and show peculiar aggregations of protoplasm at the periphery.   Protoplasm rapidly converted into peculiar globular highly refractile bodies, particularly on glucose agar. Shadow and transparent forms appear early.   Spores central, excentric or sub-terminal, oval to cylindrical, measuring usually 0.75 to 1.125 by 1.5 to 2 microns.   Spores vary greatly in shape, sometimes round, sometimes rectangular, often reniform. Growth on solid media as thick pultaceous mass, on fluid media as turbidity with little or no scum formation.

Represented by

Bacillus megatherium De Bary.
Bacillus petasites Gottheil.
Bacillus ruminatus Gottheil.

Group VII.   Round terminal spored group

Small actively motile organisms measuring 0.5 to 0.75 by 1.5 to 3 microns, often forming long threads in old cultures.   Protoplasm homogeneous.   Spores subterminal or terminal, round, thicker than the organisms from which they spring, measuring 1 to 1.5 microns in diameter.

Represented by

Bacillus pseudotetanicus (Kruse) Migula.
    (Bacillus pseudotetanicus var. aerobius Kruse.)
Bacillus fusiformis Gottheil.

Group VIII.   Cylindrical terminal spored group

Small thin actively motile organisms measuring 0.375 to 0.5 by 2.5 to 4 microns.   Slightly larger on glucose agar but no change in character of protoplasm.   Spores terminal, cylindrical, measuring usually 0.75 by 1.125 to 1.5 microns.

Represented by

Bacillus circulans Jordan.
Bacillus brevis Migula.
Bacillus terminalis Migula.

Group IX.   Central spored group

Long, actively motile organisms with pointed ends measuring 0.375 to 0.5 by 1.125 to 4 microns.   Slightly larger on glucose agar, but no change in character of protoplasm.   Spores develop in the middle of the rods which become spindle-shaped. Spores large cylindrical measuring 0.625 to 0.875 by 1.125 to 1.5 microns.

BACILLUS CENTROSPORUS nov. sp.

BACILLUS LATEROSPORUS nov. sp.

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PLATE 1

FIG. 53

FIG. 54

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
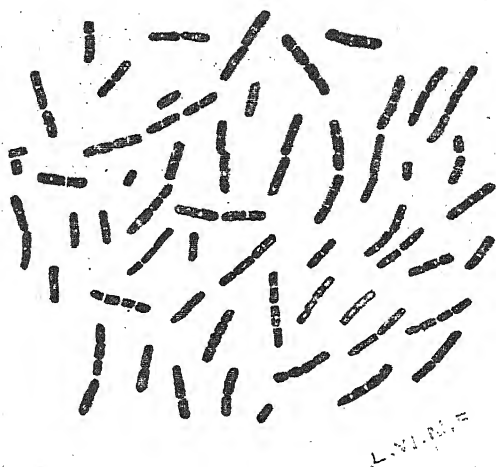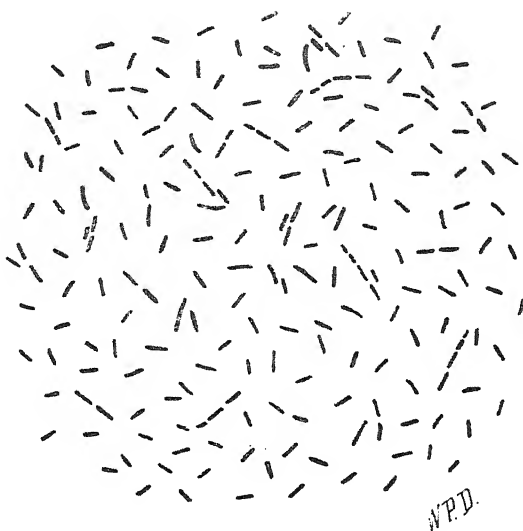
FIG. 55
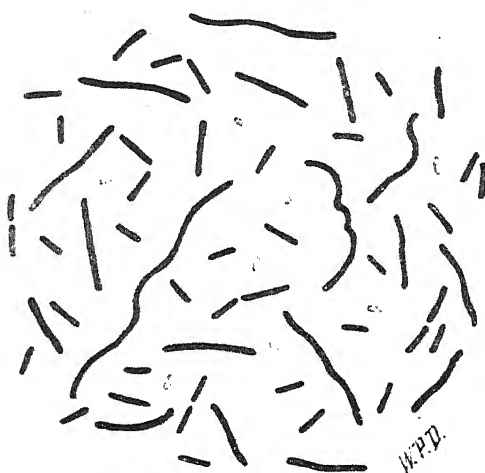
FIG. 56

(Laubach, Rice and Ford; Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG 57

FIG. 58

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

PLATE 4
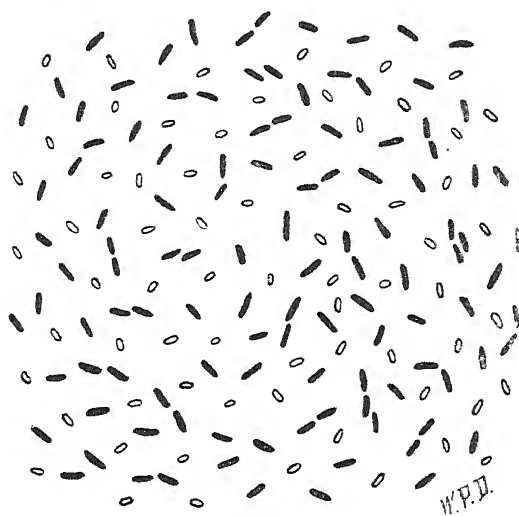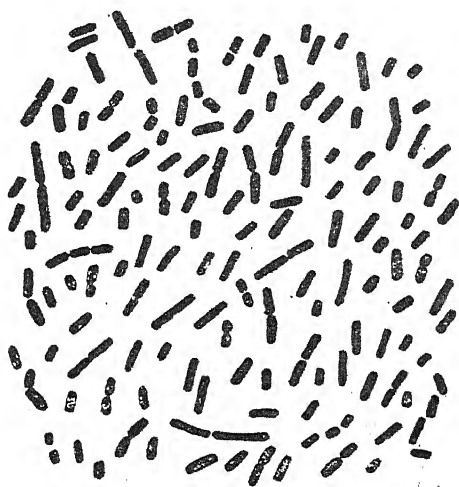
FIG. 59
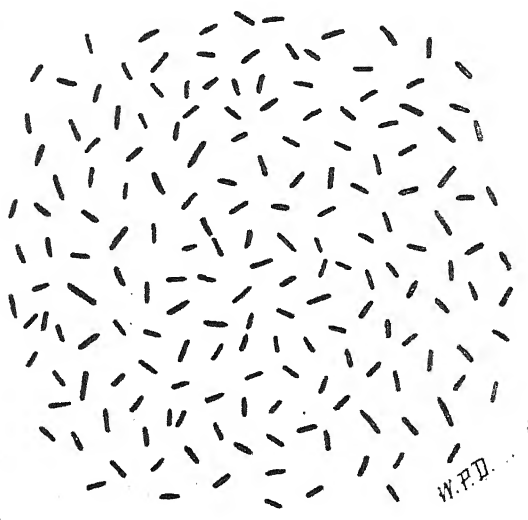
FIG. 60

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

Fig. 61
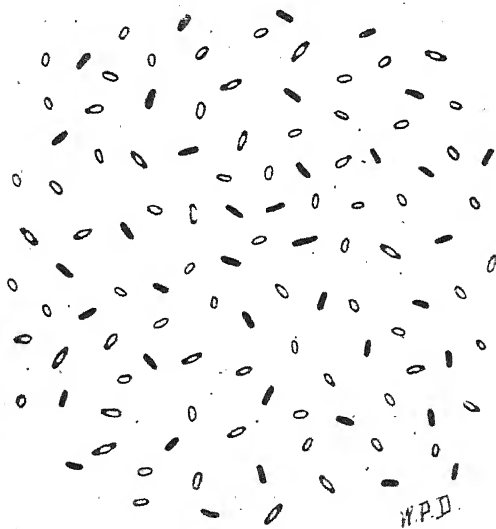
Fig. 62

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

Fig. 63

Fig. 64

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
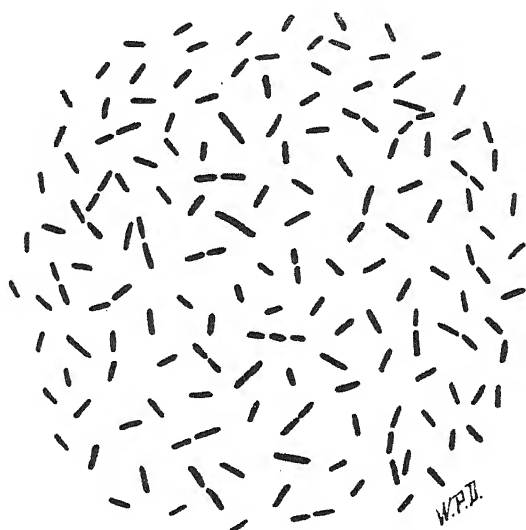
Fig. 65
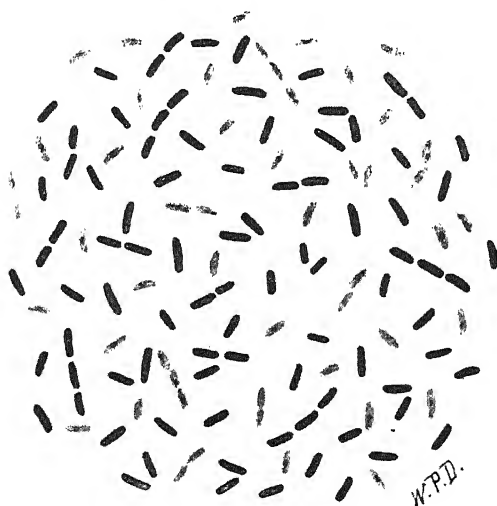
Fig. 66

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
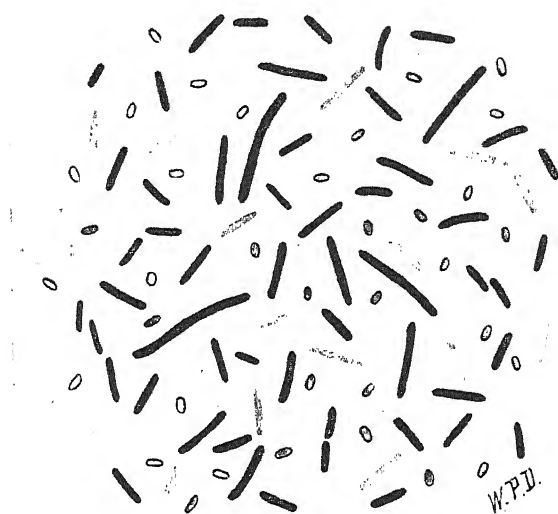
FIG. 67
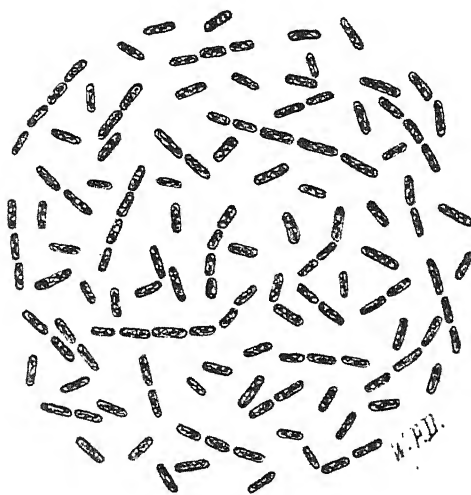
FIG. 68

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 69

FIG. 70

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

PLATE 10

Fig. 71

Fig. 72

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

PLATE 11
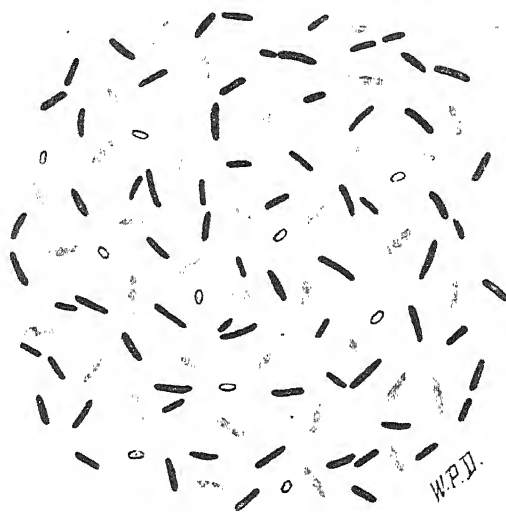
Fig. 73
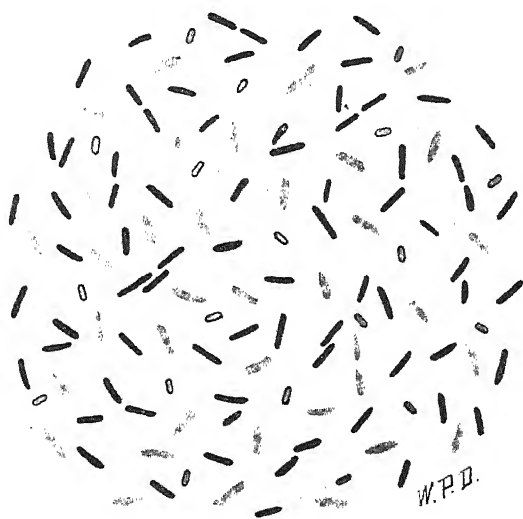
Fig. 74

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

PLATE 12

Fig. 75
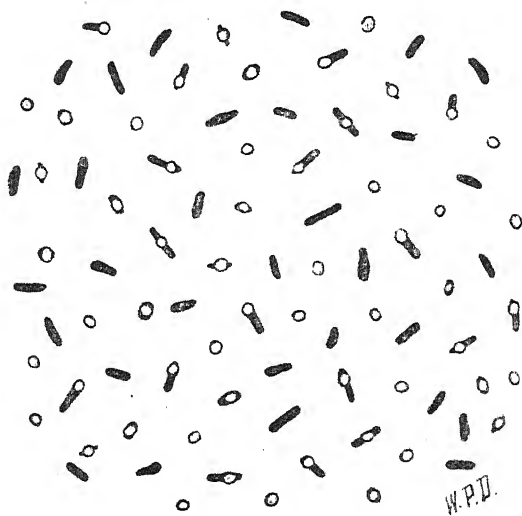
Fig. 76

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
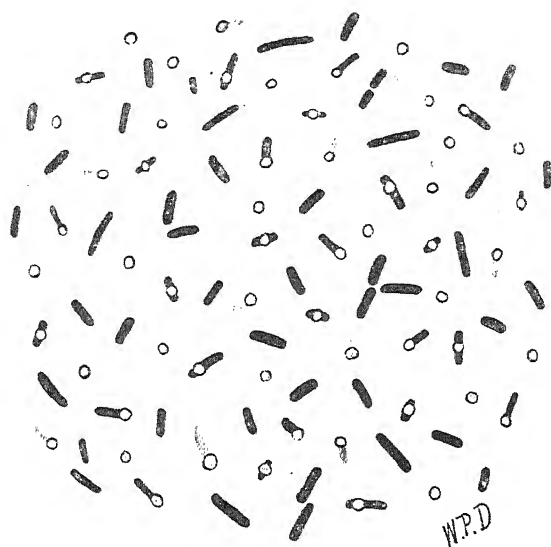
FIG. 77
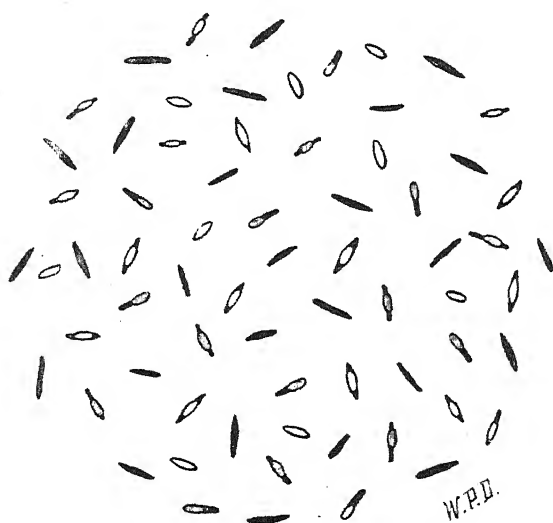
FIG. 78

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
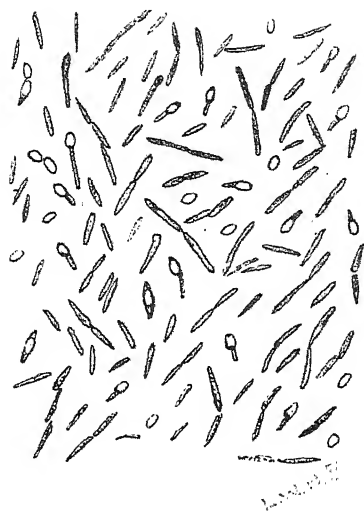
FIG. 79
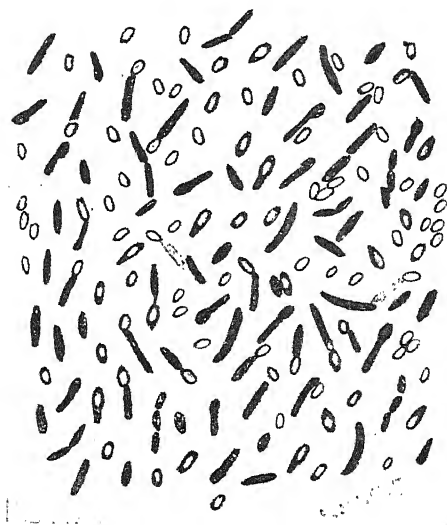
FIG. 80

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)
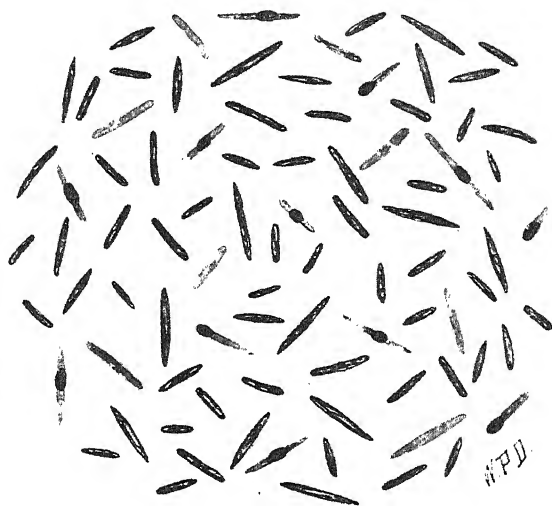
FIG. 81
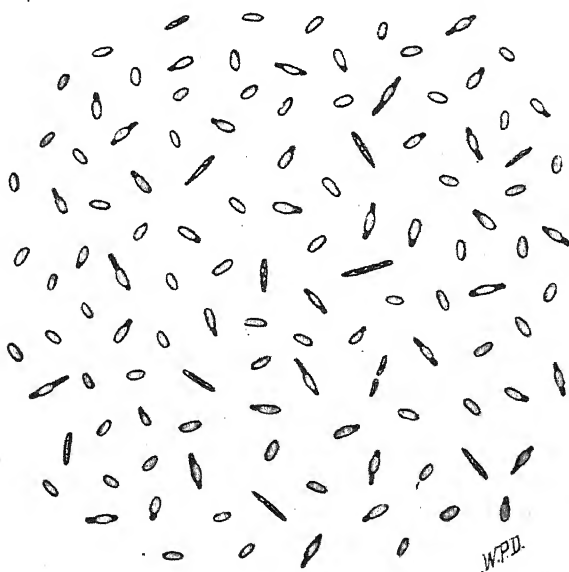
FIG. 82

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

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Fig. 71. Bacillus laterosporus from water. Plain agar, 6 hours
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A RAPID AND SIMPLE INDOL TEST

PAUL R. CANNON

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It is well known that tryptophane in an inorganic solution furnishes an excellent medium for the demonstration of indol production by bacteria. Zipfel (1) in 1912 found that indol could be demonstrated by this method at the end of twenty-four hours, giving the best reaction with p-dimethyl-amido-benzaldehyde. The test is made by adding to the culture to be tested one cubic centimeter of a solution consisting of p-dimethyl-amido-benzaldehyde, 4 parts; absolute alcohol, 380 parts; and concentrated hydrochloric acid, 80 parts.

The expense of preparing tryptophane and the great difficulty in obtaining it at all in the United States at the present time are drawbacks to the introduction of the tryptophane method. As a substitute, I have found that hydrolyzed casein can be used. Cow casein contains about 1.5 per cent tryptophane, which may be obtained in its amino-acid form by hydrolyzing the casein.

The method is as follows: 10 grams of casein are hydrolyzed by 200 cc. of 10 per cent sulphuric acid, the mixture being kept on the water bath for twenty-four hours. At the end of this time, the casein is completely dissolved and the solution is a dark brown. Next, the solution is neutralized by the addition of saturated barium hydrate, thus precipitating out the sulphate. The resulting solution is then evaporated until the amino-acids crystallize. Half of the crystalline mass is dissolved in 500 cc. of Zipfel's inorganic solution consisting of Asparagin and Ammonium lactate, 5 grams each; Potassium acid phosphate, 2 grams; Magnesium sulphate, 0.2 grams; and distilled water, 1000 grams. The medium is tubed and sterilized. Assuming that there are 0.15 grams of tryptophane in the cow casein, tryptophane should be present in the mixture to the extent of about 0.03 per cent.

Tubes of media prepared in this way were inoculated with known indol-forming bacteria, and, at the end of eighteen hours, the indol test was made by using p-dimethyl-amido-benzaldehyde. A pronounced red color almost instantly appeared, showing the presence of indol. The control and non-indol-formers remained a straw color after the addition of the aldehyde. It proved unnecessary in my tests to use amyl alcohol to dissolve out the color, although this may be done in case indol formation is doubtful.

The brief time—eighteen to twenty-four hours—necessary for the test by the above method, is a great improvement over the old standard peptone test, which required five days. Furthermore, hydrolyzed casein can easily be obtained or prepared, and the constituents of the inorganic solution are available in most laboratories.

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BACTERIAL NUTRITION: A BRIEF NOTE ON THE PRODUCTION OF EREPSIN (PEPTOLYTIC ENZYME) BY BACTERIA

NATHAN BERMAN AND LEO F. RETTGER

From the Sheffield Laboratory of Bacteriology and Hygiene, Yale University

In a recent publication (1) the authors stated that gelatin-non-liquefying bacteria of the *Bacillus coli* type are able to exert but little, if any, proteolytic action on Witte's peptone and on partially purified proteoses. The biuret test was employed in the earlier experiments, and the results were not expressed in mathematical terms. In later studies the changes in the protein content of the media have been recorded in definite figures. Furthermore, the Sörensen method for the determination of amino acids has been used along with the biuret test.

The published results in a few instances gave unmistakable evidence of a reduction in the amount of biuret-giving substances in the media containing Witte's peptone, when *B. coli*, *B. typhi* and *B. pullorum* were employed. These reductions were only slight, however, and were noticeable only after two weeks' incubation. While the changes in the protein content of the media suggested the possibility of ereptic enzyme action, no definite conclusion was warranted. Subsequent investigation has, in a measure at least, clarified the situation.

In a medium containing 1 per cent peptone, 0.25 per cent Liebig's meat extract and 0.5 per cent of sodium chloride *B. coli*, *B. typhi*, and *B. paratyphi A*, under optimum cultural conditions have slowly acted upon the biuret-positive substances in the peptone. While at least two weeks were usually required to produce any appreciable decrease in the "protein," the loss amounted to from 10 per cent to 40 per cent of the original when the digestion was allowed to continue for at least three weeks.

It was impossible to bring about a decomposition of more than

40 per cent of the original "protein" in the peptone solution, even under the most favorable conditions, as for example the addition of ammonium sulphate, asparagine, glycerine, phosphates, etc. In some instances 10 to 20 per cent marked the maximum reduction. When other peptones were employed, however, namely five different American brands, reductions considerably in excess of 40 per cent of the protein were frequently obtained. In all of these experiments the Sörensen method gave corroborative results.

Media containing Witte's peptone which had been dialyzed for at least two days underwent less change in their protein content than the corresponding solutions of untreated peptone. Furthermore, the so-called "proteose fraction" obtained from Witte's peptone by precipitation with ammonium sulphate, repeated precipitation with sodium sulphate, and prolonged dialysis, was apparently unattacked when used in place of the original commercial peptone. The results obtained thus far indicate that the gelatin-non-liquefying organisms of the Coli-typhi-paratyphi group are unable to utilize or in anyway affect the proteose portion of commercial peptones.

In the light of the researches of Emil Fischer and his pupils (2) on polypeptides, the deportment of the *Bacilus coli* type of gelatin-non-liquefying bacteria towards commercial peptone may be explained as follows. These peptones, far from being relatively simple, are mixtures of many organic substances of varying composition and complexity, particularly amino acids and polypeptides. Some of these polypeptides are in all probability quite simple, as for example the condensation product of two simple amino acids, and if we accept the conclusions drawn by different investigators, others are of a much more complex type, and are tied up with the peptone and proteose molecules. Some of the polypeptides give a positive biuret reaction, even the relatively simple ones, while others are negative to this test. All may on decomposition yield ammonia, amines and amino acids.

The proteose fraction of commercial peptone, it may be assumed, contains the relatively complex polypeptides, and hence

is less readily attacked and utilized as food by bacteria than the remainder of the mixture. On the other hand, the real peptone fraction is undoubtedly a more heterogeneous mixture of polypeptides, some of which are indeed quite simple, and are easily broken up by bacteria or their enzymes. In the proteolysis which takes place slowly under most favorable conditions, the simpler polypeptides are attacked. When these have been exhausted so-called "peptolysis" ceases.

The above explanation appears all the more probable from the fact that as bacterial decomposition continues there is a change in the biuret-giving ingredients of the peptone which is indicated by a difference in the color obtained. While the original peptone medium gives a pink color, the reaction changes if there is appreciable peptolysis, and the color becomes distinctly violet and indistinguishable from the biuret color obtained with proteoses and some of the higher proteins.

The peptolysis which is brought about by *Bacillus coli* and its close allies is undoubtedly the result of an ereptic enzyme (erepsin). This enzyme differs, however, from erepsin of animal origin in that it does not attack casein. In comparison with intestinal erepsin, and with the proteolytic enzymes of *B. subtilis*, *B. prodigiosus* and other strong gelatin-liquefying bacteria, in so far as peptolytic action is concerned, the erepsin elaborated by the Coli-typhi-paratyphi group of organisms is decidedly weak.

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A PRACTICAL METHOD FOR THE IDENTIFICATION OF GUINEA-PIGS UNDER TREATMENT

A. PARKER HITCHENS

From the Mulford Biological Laboratories, Glenolden, Pennsylvania

The problem of identifying guinea-pigs under treatment is a simple one if only a few animals are in use; the popular method of recording their colors may be adequate; or, separate cages or pens may be provided for each animal. But in laboratories where ten or more animals are inoculated every day a more highly systematized method is required.

Celluloid or aluminum ear tags are both convenient and satisfactory for a small number of animals; they are not easily lost unless two or more full grown male pigs are kept in the same pen. The ear tag method, however, has the disadvantage of consuming considerable time. Furthermore it involves an extra item of stock to look after; if the tags happen to be all used, there may be some difficulty and annoyance in finding a substitute.

Another method in common use is a pictorial description of the animal. On the record sheet is printed the outline of a guinea-pig with the regions of the body indicated. The colors are then noted on the diagram according to their regional distribution. In some laboratories, a rubber stamp is used as the basis for the pictorial description, an arrangement which may be desirable when the same record sheet is to be used for other animals. Many laboratory workers merely draw an elongated oval with little marks at one end to represent eyes and ears and record on this simple diagram the color distribution. All these pictorial methods are reliable, but so much space is required for the *esquisse* that the record of a large amount of work becomes extremely bulky.

The most valuable method for practical purposes would seem to be one whereby (1) ears tags and other equipment are rendered unnecessary, (2) the description is recorded on a single line, on ordinary paper without special ruling or diagram. Although systems similar to the one about to be described have probably been in use for some time, none has appeared to the writer so satisfactory as the elaboration of what was originally merely a makeshift series of abbreviations invented at the time for a particular purpose. The fundamental idea of the plan in question was conceived by Dr. Joseph McFarland more than fifteen years ago; and with a few modifications by the writer, the same method has been used in these laboratories ever since that time. The readiness with which an assistant of no training can learn to describe and recognize guinea-pigs accurately seems to be sufficient recommendation for a more widespread use of this system of abbreviations.

The rules according to which guinea-pigs are described and identified in the Glenolden laboratories are as follows:

IDENTIFICATION OF GUINEA-PIGS

1. Not more than 5 to 8 guinea-pigs are kept in one pen.

2. The assistant in describing the animals mentions first the weight, and then the sex, before calling out the description.

3. The sex is denoted thus:

Male.. ♂
Female.. ♀

4. The sides (Right and Left) and the colors are represented in abbreviations by the capitalized initial letter.

The sides of the animal are written thus:

Right.. $\mathcal{R}$.[1]
Left... $\mathcal{L}$,

The colors are recorded as follows:

[1] The difference between the abbreviations for red, " $\mathcal{R}$ " and right, " $\mathcal{R}$.", are to be noted.

White.. *w*
Cream... *b*
Yellow.. *y*
Silver (silver agouti).. *S₂*
Silver and yellow... *sy*
Red... *R*
Red and Gold.. *Rg*
Gold (golden agouti).. *g*
Fawn (also chocolate)... *F*
Yellow and Slate.. *yS*
Slate... *S*
Red and Black... *RB*
Black... *B*

5. The varieties of coat are described thus:

Hair Clipped .... **C**
Rough... *Ro*
Angora .... *go*

6. The regions of the body are represented by small letters as follows (see fig. 1):

	Singular	*Plural*
Nose..	*n*	
Face..	*e*	
Head..	*d*	
Eye...	*i*	*ii*
Ear...	*r*	*rr*
Root (of ear)..	*√*	*√*
Shoulder...	*s*	*is*
Pleura...	*p*	*pp*
Hip..	*h*	*hh*
Flank (pleura and hip)...................................	*f*	*ff*
Sacrum...	*x*	

7. The pigs are described in the order of the depth of their coloring, a white pig, for instance, is mentioned first, then a pig with few markings over only a small part of the body. Pigs of solid colors come last in the order given above. White is mentioned only when the entire pig is white.

8. In describing an animal, the assistant starts with the fore part of the body on the right side (R); he mentions the color— say, of the right eye—and proceeding backward enumerates the

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[2] Note the difference between "s" (silver) and "s" (slate).

parts having this color, first on the right side then on the left (provided, of course, this color is distributed on both sides); he then mentions the next color in the same order from the head backward.

9. In the case of two pigs with mixed colors over the entire body it is convenient to note the two colors and follow this by a characteristic difference. Occasionally there may be several pigs

Figure I.
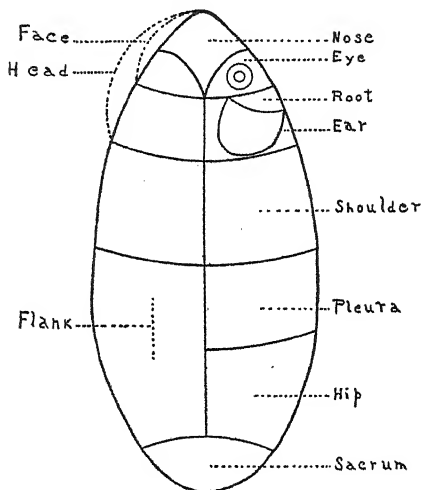
exactly or almost exactly alike—white, for instance, or red and black. In such cases, it may be necessary to clip the hair on the nose or some other part of the body.

10. The description of 8 guinea-pigs in a certain pen might be written as follows:

w	......White
RRi	......White with red about the right eye
SReLiLyRHp	......Slate right ear and left eye and hip, yellow right shoulder and pleura
BRnPr	......Black eyes, ears and red pleura
RBRn	......Red and black mixed but with red on nose
RBBn	......Red and black mixed but with black on nose
RBCn	......Red and black mixed with the hair on the nose clipped
B	..... Black

It will be noted that the entire description of the animal is recorded without raising the pen or pencil from the paper.

Since this system is based upon abbreviations, the question may be asked, why not go still further and use shorthand characters? It is true that shorthand once learned would consume less time, but it would require considerable training for a new assistant to become sufficiently familiar with shorthand characters to write them rapidly and accurately. In the author's experience, on the other hand, it has not required more than two or three fifteen-minute periods to instruct any new assistant in the satisfactory use of the above method.

A NOTE ON THE PREPARATION OF AGAR AGAR CULTURE MEDIA

C. L. WILLIAMS AND H. P. LETTON

United States Public Health Service

One of the greatest troubles in preparing agar agar is clearing the medium. Filtering through paper is very slow and requires a special water jacket for the filter in order to keep the agar hot enough to prevent solidification. Nor does a paper filter entirely clear the agar. The more usual method of filtering through cotton requires repeated filtration, and even then seldom gives a perfectly clear product.

The principal cause of failure is apparently the presence of finely divided insoluble particles derived from the agar agar, which become evenly distributed through the medium after this substance has been dissolved. These particles are often so fine that a great part of them will pass even through filter paper.

In the laboratory of the Sanitary District of the Great Lakes various methods were tried to facilitate filtration while securing a clear product. The use of egg albumin as a coagulant in some media suggested coagulation as probably successful, and the use of the Hygienic Laboratory method of preparing Endo's medium gave us the clue to the present procedure.

In the Hygienic Laboratory method for Endo, the medium is partially cleared by pouring into large beakers while hot, where it is allowed to cool and harden. On inverting the beakers the solid mass of agar slips out, and the bottom where most of the suspended matter has settled is cut off and wasted. This process was modified by us in the case of ordinary agar media by placing the container in a large water bath (a large saucepan usually served for this) which was kept over a low flame for several hours, usually over night. This procedure caused the collection of the suspended matter into flocculent masses which settled to

the bottom. The result was more than a settling of the solid particles, it was rather the ensnaring of these in a delicate coagulum, much like the coagulum of aluminum hydrate and like that substance in requiring some time for its action. After the formation of this flocculent precipitate a single filtration through cotton produces a beautifully clear filtrate.

The following points should be remembered. Any stirring before filtration will quickly break up the coagulum, which is very delicate, a cloudy filtrate resulting. For this reason, the reaction should be adjusted before placing on the water bath, as we have found that this changes very little during its stay there. After sterilization in the autoclave the medium remains clear if soon cooled. If remelted for pouring plates it is clear at first, but after standing some time in the water bath a second flocculent precipitate forms, which however settles to the bottom so that with careful handling the clear supernatant medium may be readily poured off.

No attempt has been made by us to review the literature dealing with this subject, so that we do not know if this procedure has been described before. We have never seen it mentioned however. Any one who has had to make any considerable amount of agar will readily understand the value of so simple a method of obtaining a clear product. We prefer this method even to clearing with egg albumin, since the result is a clearer medium although it takes more time to prepare.

BOOK REVIEWS

A Text-book upon the Pathogenic Bacteria and Protozoa. For Students of Medicine and Physicians. By JOSEPH McFARLAND, M. D. Eighth edition, thoroughly revised. Octavo of 807 pages with 323 illustrations, a number of them in colors. Philadelphia: W. B. Saunders Company. 1915. Cloth $4.00 net.

The author opens with an excellent historical introduction, tracing the evolution of bacteriology through three stages—biologic contributions, chemical contributions and the modern medical and surgical contributions. The first part of the book, headed "General," is devoted to a discussion of the biology of microörganisms, methods of studying them, and infection and immunity. Chapter I—on Structure and Classification of Microörganisms—includes bacteria, higher bacteria, yeasts, molds and protozoa. No mention is made of the chemical composition of bacteria. The nature of the cell substance is passed over in one sentence. The author does not take up the bioligoc significance of spores, the conditions under which sporulation takes place, and conditions under which germination occurs. The classification of bacteria is treated very briefly, Migula's tables being given, while Jensen's system does not appear. Only ten pages are given over to a discussion of these subjects, about one-third of space allotted in other books.

Chapter II (pages 50–65), on Biology of Microörganisms is too compact and synoptic in its introductory discussion of biologica relations of bacteria to temperature. Mere definitions of minimum, optimum and maximum temperatures ove no significance without some discussion of the thermal death points of various organisms, range of temperature at which growth takes palace among different types, etc. Similarly more than one page should be devoted to a discussion of the food supply of bacteria, and the synthesis affected by nitrifying bacteria should be included under such a topic

Chapters III and IV (pages 66–143) on Infection and Immunity are clearly presented. The Lateral Chain Theory of Ehrlich is explained by a lengthy quotation from his Croonian Lecture before the Royal Society of London. A section in this chapter is devoted to Defensive Germents with a description of the Abderhalden Reaction and the technique involved in making the test. Staining and observation of protozoa, and bacterio-vaccines and their preparation are some of the new topics treated.

Chapters XI, XII and XIII (pages 234–248) devoted to Bacteriology of Water, Air and Foods are necessarily brief and incomplete.

In Chapter XVII (pages 251–262) on Determination of the Value of Antiseptics, Germicides and Disinfectants, a detailed description of the technique of the Anderson-McClintic method is given.   The important theoretical work of Chick is not mentioned in this chapter.

Part II is devoted to a discussion of the infectious diseases and the specific microörganisms—each disease being treated in a separate chapter,—and this Part is very fully and well treated.

It is curious that the importance of septic sore throat as a new disease should not find a place in a discussion of streptococci.   Cole's work on the Pneumococci has not yet been included in the chapter on Pneumonia.

Chapters XIX (pages 471–493) on Malaria and Chapter XX (pages 494–505) on Sleeping Sickness treat these subjects in much detail.

There is no mention of Texas Tick fever or Rocky Mountain Spotted Fever.   Such diseases as measles, scarlet fever, foot and mouth disease and mumps should find a place in a chapter on filterable viruses, which the book does not contain.

On the whole the text presents the subject very clearly, and the illustrations are exceedingly good.   The author in the preface says that the book is a medical work intended for medical students and practitioners; and its purely medical aspects are excellently handled.   It is unfortunate however, that medical students should not master the undelying fundamental biological principles of bacteriology in their widest aspects for these are of the utmost importance in their application to modern preventive medicine.

D. GREENBERG.

The Principles of Pathologic Histology.   By FRANK B. MALLORY, M.D., Associate Professor of Pathology, Harvard Medical School and Pathologist to the Boston City Hospital.   Octavo of 677 pages, with 497 figures containing 683 illustrations, 124 in colors.   Philadelphia and London: W. B. Saunders Company, 1914.   Cloth $5.50 net.

This interesting and useful book on pathologic histology reflects admirably the experience of its well-known author.   It is confessedly individualistic and contains practically no direct reference to the work and opinions of others.   The author is undoubtedly right in insisting that the study of the lesions themselves is the only proper source of a balanced judgment in reference to the terminal aspects of disease.   It seems a pity, however, that on controversial points he has not seen fit to state both sides of the question and then give his own interpretation.

The book may be reviewed from the standpoint of pathology in the larger sense, and again from the standpoint of the morphological aspects of disease.   From the latter viewpoint it is to be praised for the orderliness of presentation and the remarkable clearness and beauty of the numerous original illustrations.   In the first part, dealing with General Pathologic Histology, the subjects of inflammation and the retro-

grade processes are treated in a masterly and deservedly extended fashion. The section on Tumors, comprising approximately one-third of the book, is a remarkable monograph which presents a summary of the author's own extensive material basis on which his mature and deservedly respected opinion is based. The treatment of this phase, important as it is from a diagnostic standpoint, seems disproportionate for the needs of beginning students of disease processes however valuable it may be for the specialist. The second part dealing with Special Pathologic Histology is on the other hand somewhat short for general needs, and lays unusual emphasis on those rarer lesions which have happened to attract the author.

From the larger standpoint of pathology as a study of the natural history of disease the book represents an unfortunately restricted attitude. In studying disease we are of course interested in studying the cause and progress as well as the result produced. In the infectious diseases of known causation the entire course of the process can be followed, particularly with the aid of animal experimentation. Much can be learned concerning the progress of a disease if an approximately complete set of lesions is gathered by the diligent collector at the autopsy table, provided always that the successive stages be subsequently fitted into their proper places. Much more rapidly and certainly can a chronological series be prepared by injecting the specific microöorganism into a suitable animal. To Mallory "recourse to animal experimentation has often served to confuse a subject rather than to simplify and clear it up." Experimentation unfortunately requires a certain type of ingenuity and the power of inductive reasoning in addition to the deductive reasoning required by the collector. The author has correctly stated that from a study of lesions alone "we are in a position to read the process (of disease) backward with some degree of certainty." It is profitable that the life work of a group of individuals should center in pathology as viewed from this essentially Chinese angle, but it is inexcusable that the work of the growing majority who choose to follow pathology forward rather than attempt to read it backward should not at least be recognized.

This failure to recognize the essentially dynamic functional viewpoint of disease has led Mallory into numerous errors in treating of the infectious diseases. Bacterial causation he recognizes in so far as bacteria may be fixed and stained, but failure to utilize or even absorb the experimental viewpoint has led to numerous unlikely hypotheses. Practically all bacteria are stated to act by the production of a "toxin" of greater or less strength as judged by the response of fixed cells; antitoxins are asserted to be formed in typhoid fever and indeed they are said to be formed in the so-called "endothelial leucocytes." It is needless to state that there is no experimental evidence for a statement of this sort. The very name of "endothelial leucocytes," for the description of which Mallory is properly appreciated, is probably inexact if we are to accept their origin from connective tissue as shown by Evans in his work with vital stains. For his insistence on the objectivity of lesions we have only to thank Mallory, but it is unfortunate that he has not employed his imagination in experimental verification of his working hypotheses.

F. P. GAY.

ABSTRACTS OF AMERICAN BACTERIOLOGICAL LITERATURE

ANIMAL PATHOLOGY

Notes on the Histo-Pathology of the Intestines in Young Chicks Infected with Bacterium pullorum. G. EDWARD GAGE and JAMES F. MARTIN. (Jour. Med. Res., 1916, **34**, 149–155.)

Typical strains of *Bacterium pullorum*, when injected into young chicks, produced death with characteristic symptoms of bacillary white diarrhea in 49 per cent of the animals. Chicks which survived the injection showed retarded growth and general weakness. The chief pathologic changes on section were marked injury to the mucosa, associated with hyperemia, hemorrhagic exudation and leucocytic infiltration.

H. W. L.

The Diptheroid Bacillus of Preisd-Nocard from Equine, Bovine and Ovine Abscesses. Ulcerative Lymphangitis and Caseous Lymphadenitis. I. C. HALL and R. V. STONE. (Jour. Infect. Diseases, 1916, **18**, 195–208.)

The authors call attention to the presence in the United States of the bacillus of Preisz-Nocard, which they isolated from horses and from a calf. They point out the uncertain significance of experimental orchitis in guinea-pigs as a test for glanders, and emphasize the necessity of microscopic and cultural examination of pus for diagnostic purposes.—P. B. H.

BACTERIOLOGY OF SOILS

Studies in Sulfofication. P. E. BROWN and H. W. JOHNSON. (Soil Science, 1916, **1**, 339–362.)

It is suggested that applications of sulfur-containing commercial fertilizers to soils will prove profitable unless different methods of soil treatment are employed than those in use at present, because at some future time sulfur will be lacking. In other words, for permanent soil fertility, the sulfur supply for crops must be considered. Chemical tests were made for determining sulfofication produced in a sulfur-rich soil to which various chemicals were added.—Z. N.

Preliminary Experiments on Some Effects of Leaching on the Soil Flora. C. B. LIPMAN and L. W. FOWLER. (Soil Science, 1916, **1**, 291–297.)

It seems that leaching affects the bacterial flora of soils profoundly. Nitrification, nitrogen-fixation and cellulose decomposition are wholly

or almost wholly checked by this process, especially if salts are present prior to leaching. Investigations are being carried on to determine if the injury done is or is not an ephemeral one which may entirely disappear in a few months under field conditions. This and other experiments in progress may lead to the discovery of the cause or causes of damage done to relatively new soils by irrigation flooding.—Z. N.

Quantitative Method for the Estimation of Bacteria in Soils. R. C. Cook. (Soil Science, 1912, 1, 153–163.)

A synthetic medium is most desirable so that results may be comparable at different times and places, and for quantitative work the medium which will permit the development of the maximum number of colonies is usually most satisfactory.

Twelve media were at first compared on four soils, Lipman and Brown's modified synthetic agar being taken as a basis for comparison, the only difference being in the methods of sterilization. In the third series of experiments seven media were eliminated and in the last two series, Lipman and Brown's agar was eliminated. Results indicate that sodium asparaginate agar, albumen agar, and urea ammonium nitrate agar will in most cases give a greater colony development for soil bacteria than other media in common use in bacteriological work. A five-day count gives much higher bacterial counts than a three-day count.—Z. N.

Stimulating Influence of Arsenic upon the Nitrogen-fixing Organisms of the Soil. J. E. Greaves. (J. Agr. Res., 1916, 6, 389–416.)

The author has previously shown that arsenic stimulates the nitrifying and ammonifying powers of soil. The present investigation is to show whether it has a similar effect upon nitrogen-fixation. Results show that all forms of arsenic tested, except paris green, stimulate the nitrogen-fixing power of soil, but not so greatly as the nitrifying power. These results are obtained only when the tests are made in soil (either untreated soil or sterilized soil reinoculated); for when solutions adapted to nitrogen-fixation are inoculated with soil, the addition of arsenic always proves toxic to the organisms concerned. One culture of *Azotobacter* was obtained that was directly stimulated by the arsenic; but in general, increase in nitrogen-fixation is observed only when a mixed flora is used. If the soil infusion used for inoculating sterile soil is previously filtered or heated as high as 55°C., no stimulation is brought about by the arsenic.

The author does not favor any one theory to account for these results; but considers the suppression of harmful microörganisms to be at least a partial explanation. He points out that the results are almost exactly what might be expected on the assumption that these harmful organisms were protozoa.—H. J. C.

The Occurrence of Bacteria in Frozen Soil. E. C. HARDER. (Bot. Gaz., 1916, **61**, 507–517, two graphs.)

Investigations were begun during the latter part of October and the samples were examined every week until the latter part of February. A dark, medium rich, slightly sandy garden soil was studied, obtained from the University campus near the College of Agriculture at Madison, Wisconsin. The soil was sampled to a depth of 6 inches each time. The entire sample was thawed when necessary and thoroughly mixed in a mortar previously washed out with 95 per cent alcohol. After proper dilution, plates were poured and counted after an incubation period of eight days at 28°C. Heyden Nahrstoff agar was used as a culture medium.

In order to determine whether the high bacterial content of frozen soil was due to an actual increase or whether other factors brought about this phenomenon, two duplicate sets of potted soils were prepared. One set was kept at room temperature and the other set was placed outside, subject to atmospheric temperatures.

Summary of results. 1. It was found that the number of bacteria in surface soil increased markedly after heavy frost, and in general maintained a high average during the winter months. The increase and decrease, however, were found to bear a distinct relation to the moisture content.

2. The potted soils failed to show such marked increase in the bacterial count after frosts. On the contrary the enriched cultures showed a distinct retardation of bacterial growth when in a frozen condition.

3. The bacterial flora was much the same during the fall, winter and spring, with the exception that after heavy frosts the small transparent colonies characteristic of water and of deeper soils, formed a larger proportion of the growth on the plates.

From these results it seems reasonable to conclude that ordinary soil bacteria undoubtedly withstand cold to a marked degree, even temperatures as low as 40°C. or more below zero. The increase in numbers, however, seems to be due to mechanical transpiration of moisture coming up from below during heavy frost, and where such transpiration is not possible there is an actual retardation in growth as compared with that in unfrozen soils.—J. T. E.

Incubation Studies with Soil Fungi. S. A. WAKSMAN. (Soil Science, 1916, **1**, 275–285.)

Too little attention has been directed to the possible influence of fungus forms as one of the important factors in soil fertility.

The longer and more complex life cycle of the fungi no doubt makes their relation to the fertility of the soil different at successive stages of growth.

Bacteriological methods were pursued in the study of three organisms which represent three very important groups of soil organisms: *Mucor plumbeus*, *Penicillium sp.*, and *Monilia sitophila*.

At first the moisture relationship and incubation period were deter-

mined for these three molds after 6, 12 and 18 days upon a culture medium composed of gravelly loam plus dried blood and cotton-seed meal as ammoniates. From results of this preliminary experiment attempt was made to determine how the biological stage affected ammonification. The periods of most active ammonification correspond to those of active spore formation for the respective organisms and the smallest amount to the time preparatory to actual spore formation. The *Monilia* shows the largest ammonia accumulation within the first 3 or 4 days; the *Penicillium*, between 10 and 15 days and the *Mucor* between 6 and 10 days.

An analogy is found in the growth of legumes like the clovers. Nitrogen is fixed during the period of active growth of the plant, the fixation ceasing almost entirely when seed formation begins.—Z. N.

Diastase Activity and Invertase Activity of Bacteria. GEORGE P. KOCH. (Soil Science, 1916, 1, 179–196.)

This paper is limited to the study of diastases and invertases produced by bacteria which are concerned in the production of nitrogen compounds for consumption by plants and in the decomposition of carbohydrates in the soil.

The author determined that sufficient diastases and invertases were secreted by bacteria so that they could be quantitatively determined and also that there was considerable variation in the enzyme (diastase and invertase) secretion by organisms developed in culture solutions of different composition. The enzyme secretion by bacteria at different periods varies from day to day under conditions otherwise the same and there seems to be no direct correlation between hydrolytic enzyme secretion by bacteria and their property of decomposing proteins. The enzyme activity of various organisms and their ability to decompose proteins vary greatly; there is also a variation in enzyme activity of different cultures of the same species. No correlation was found between the secretion of enzyme and the decomposition of proteins by bacteria, the property of the cultural solution to rotate the plane of polarized light, the percentages of reducing compounds, the formation of acid or the numbers of organisms.

The rotatory power of a solution may be increased as well as decreased by bacteria; they do not produce a surplus of reducing compounds. Bacteria also seem to have the property of causing a condition which will prevent starch hydrolysis and sucrose inversion. There may be a possible correlation between the protein decomposition determined as ammonia and the formation of acid.—Z. N.

Bacterial Numbers in Soils at Different Depths, and in Different Seasons of the Year, SELMAN A. WAKSMAN. (Soil Science, 1916, 1, 363–380.)

Four soils were studied. Meadow soil gave the largest bacterial counts at a depth of 1 inch, the 1-inch layer of this soil being richer also in organic matter and nitrogen content than that of the garden and orchard soils. The forest soil, though showing a high carbon and

nitrogen content gave the lowest bacteria counts probably because of the high acidity and large amount of undecomposed matter. The numbers of bacteria in the soils studied were not governed either by the moisture content of the different soils, or by the nitrogen and carbon contents.

The greatest number of bacteria were found at a depth of 1 inch in the soils that are under shade the year around. The garden soil gave on the average the largest numbers 4 inches from the surface. The numbers of organisms decreased regularly from a depth of 1 inch down to a depth of 30 inches.

Three soils showed a regular decrease in the lime requirement down to 30 inches and in all soils this was accompanied with a more or less gradual decrease in the nitrogen and carbon content.

Frozen soil, though showing a high bacterial content, did not give the largest bacterial numbers found through the year.—Z. N.

The Inoculation and Incubation of Soil Fungi. NICHOLAS KOPELOFF·
(Soil Science, 1916, **1**, 381–403.)

The fungi employed in these studies were isolated from soil on the College Farm. They were identified as *Rhizopus oryzae* (Wendt), *Zygorrhyncus vuilleminii* (Namyslowski), *Rhizopus nigricans* (Ehrenberg), *Penicillium sp. 10*. Spores of these fungi were obtained for use by growing them on Cook's No. II fungi medium. Suspensions of a known number of spores of each fungus were added to 100 gm. portions of soil of an optimum moisture content to which dried blood and cottonseed meal were also added. Ammonia determinations were made at the end of 7 days.

An increase in the number of fungus spores inoculated into the soil was found to be related to a proportional increase in ammonia accumulation but this increase was not proportional beyond a certain limit.

Under the conditions of moisture and temperature employed, cotton-seed meal appears to be the more acceptable source of food for the organisms studied. A 7-day incubation period may be recommended as most desirable for the study of soil fungi other than those belonging to the Penicillium group.

A striking increase in ammonia production was observed to take place every *other* day (after the first five days). This leads to the conclusion that the production of ammonia is dependent on the metabolic processes of the fungus rather than the biological stage of spore production and germination—Z. N.

Studies on the Decomposition of Cellulose in Soils. I. G. McBETH.
(Soil Science, 1916, **1**, 437–486.)

Detailed directions are given for the preparation of cellulose for cellulose agar. The action of cellulose-dissolving organisms was studied not only on typical cellulose such as is found in filter paper or in cotton fiber but on the cellulose of plant tissues freed from encrusting substances. The method for preparation of this plant cellulose is given in detail.

The cellulose-dissolving bacteria isolated from soils by means of the cellulose agar plate method, have the power of dissolving the cellulose of alfalfa. Twenty-five species of cellulose-dissolving bacteria dissolved alfalfa cellulose as readily as that prepared from filter paper.

Thirty-six species of this type of bacteria have been isolated and their cultural and morphological characteristics are given in detail. Fifteen new species are included.

A provisional key for identifying and comparing species of bacteria which dissolve cellulose is appended.

Many of the data called for by the card of the Society of American Bacteriologists seem to have little significance in the separation of members of this group.

Filamentous fungi and actinomyces also unquestionably play an important rôle in the destruction of cellulose in nature.—Z. N.

Actinomyces of the Soil. SELMAN A. WAKSMAN and ROLAND E. CURTIS. (Soil Science, 1916, **1**, 99–135, 3 plates.)

Seven different types of soils from different localities and under differing climatic and cultural conditions were used. Brown's albumen agar, slightly modified, was used for isolation of the actinomyces from the soil and for counts. Each organism isolated was studied on Czapeck's solution, agar, potato, and on 15 per cent gelatin in distilled water.

All the actinomyces studied liquefy gelatin and they may be divided into two groups depending on their differences in color production, one browning the liquefied portion, the other remaining colorless. A characteristic of some species is the production of an aerial mycelium on gelatin.

The numbers of actinomyces decrease with soil depth, but their numbers, relative to those of bacteria and fungi, increase.

Thirty species of actinomyces are classified, and their morphology and cultural characteristics on the above media are considered in detail. A key is included facilitating their identification.

The average optimum, maximum and minimum temperatures are 30°C., 50°C. and 15°C. respectively. The actinomyces do not play any appreciable rôle in the soil as ammonifiers; they readily assimilate NO_2, NO_3, NH_3 and organic compounds of nitrogen and characteristically reduce nitrate to nitrite, but not to free nitrogen or NH_3.

Actinomyces are strong cellulose decomposers and this fact in combination with their weak ammonia production leads the author to think that the probable rôle of the organism in the fertility of the soil lies in the formation of humus. Therefore, in arid soils where cellulose destruction has been found to be extremely rapid, actinomyces should be expected in abundance.—Z. N.

Some Factors that Influence Nitrate Formation in Acid Soils. E. B. FRED and E. J. GRAUL. (Soil Science, 1916, **1**, 317–338, 1 pl.)

Acid soils do not possess a strain of nitrifying bacteria especially resistant to soil acidity. The nature of the compound to be nitrified

plays an important part. For example, in acid soils organic nitrogen nitrifies much more rapidly than nitrogen from ammonium sulphate; the reverse is true in non-acid soils.

In soil treated with calcium carbonate there is an enormous multiplication of the nitrate bacteria. At first, one or two weeks after treating, calcium carbonate stimulates nitrate formation, later the reverse is true. In the presence of organic nitrogenous substances as casein and gelatin, calcium carbonate did not permanently increase the accumulation of nitrates.

All soils stored under conditions that prevent leaching showed a gain in nitrate nitrogen.

Considering the data given, as a whole, it seems that under laboratory conditions the beneficial effect of $CaCO_3$ on plant growth must be accounted for by some processes other than the direct effect on nitrification as this effect of $CaCO_3$ on nitrification takes place before higher plants begin to draw heavily on the nitrogen of nitrates. Moreover, the period of rapid accumulation from liming may result in a loss of nitrogen from leaching of the nitrates. These questions can be determined only by field tests.—Z. N.

The Influence of Some Common Humus-Forming Materials of Narrow and of Wide Nitrogen-Carbon Ratio on Bacterial Activities. P. E. BROWN and F. E. ALLISON. (Soil Science, 1916, **1**, 49–75.)

Ammonification, nitrification and azofication were found to be increased to a considerable extent by application of the common humus-forming materials (dry) in maximum amounts for farm conditions; horse-, cow- and rotted manures, oat straw, timothy-, cowpea- and clover hays and corn stover. In general the manures favored and the legume hays depressed ammonification. Increases in ammonification were independent of the N-C ratio of the materials added, and were probably dependent on the chemical composition of the substances. If the humus-forming materials especially the manures, had been applied undried as under field conditions, ammonification would have been accentuated.

In contrast, nitrification was increased by leguminous green manures, and retarded somewhat by animal manures. These increases were apparently independent of the N-C ratio in the substances.

Azofication was favored by manure, straw stover and non-leguminous hays, and leguminous hays in order, the N-C ratio of these materials being of little or no significance as indicated by their effects on azofication.

Indications were, however, that non-legumes and straws might increase azofication on soils to a large enough extent to make their use more profitable than that of legumes which add nitrogen to the soil, but are somewhat more expensive to use.

The substances with wide N-C ratio decreased the crop yield while those of narrow ratios gave increases. The N-C ratio was found to be of more importance in determining the effect on the second crop of oats than on the first crop.

To increase azofication, non-leguminous manures must remain longer in the soil than leguminous in order to give time for considerable decomposition to occur before a crop is grown to test the effects.—Z. N.

Can Soil Be Sterilized without Radical Alteration? DAVID A. COLEMAN, H. CLAY LINT, and NICHOLAS KOPELOFF. (Soil Science, 1916, 1, 259–274.)

An effort was made to devise some method whereby soil might be rendered sterile with a minimum amount of alteration. Four different lines of experimentation were carried on: 1. The intermittent sterilization of soil by dry heat; 2. Various chemical substances used as soil antiseptics; 3. Volatile antiseptics applied in partial vacuum; and 4. Volatile antiseptics applied under pressure at 80°C.

Both moist and dry soils were sterilized at 82°C. for 1 hour on 1 to 5 successive days. Ammonia determinations were made after each heating, also bacterial counts on Lipman and Brown's synthetic agar. All protozoa were killed at the first heating; species of *Penicillium* and *Mucor* persisted throughout. The numbers of bacteria in the moist soil decreased from 47,750,000 per gram on the first day to 1,500 on the last day while although there was an initial depression in numbers of bacteria in the dry soil, an increase occurred on the first day, then a gradual decrease only. This method of soil sterilization is decidedly more efficacious in the sterilization of moist than of air-dry soil. It increases the total solids in the soil about 46 per cent, which is only one sixteenth as much as by the common method of steam sterilization. Where the time element is of considerable importance this method is undesirable.

As soil antiseptics, were used 1 per cent (on the basis of 100 gm. of air-dry soil) of ethyl alcohol, ethyl ether, toluene, carbon bisulfid, chloroform and hydrogen peroxid. Chloroform caused a decrease of 86 per cent of the original bacterial content and caused the least alteration in the chemical constitution of the soil; carbon bisulfid and toluene were next in order.

In addition to the first four volatile antiseptics named above, osmic acid was used in the third method of sterilization. The antiseptic vapor was allowed to remain in intimate contact with the soil for $1\frac{1}{2}$ hours for 3 successive days. Carbon bisulfid, toluene and ethyl alcohol, in order, caused a decrease of 99+ per cent of the original soil flora, although their action is more efficient in air-dry soil.

Carbon tetrachloride, carbon bisulfid, ethyl ether and chloroform were used in the heat plus pressure method. They were used at a temperature of 80°C. for 3 successive days upon moist and air-dry soil. The decrease in bacterial flora, in general, approximated 98 per cent. Carbon bisulfid was the only chemical which proved superior to the check treatment; it developed a pressure of 20 pounds. Ethyl ether which was least efficient developed only 6 pounds. A possible correlation might be obtained between the pressure developed during treatment and the effectiveness of the sterilizing agent. Carbon tetrachloride, which developed 5 pounds pressure is an exception.—Z. N.

Studies on Soil Protozoa. Selman A. Waksman. (Soil Science, 1916, 1, 135–153.)

The author found that no flagellates could be found in a living condition before sixteen minutes and no ciliates until after sixty-two minutes in a soil which was covered with water during these periods. The longer time allows a closer observation among the soil particles and a more thorough examination of the field. There is no doubt that there is a minimum moisture content for each soil below which protozoa cannot be found in a living condition.

Four soils were selected which varied in humus content and were under crops or orchard. Examinations were made every day for ten days in succession during which period several rains occurred. The moisture content proved to be a limiting factor but not the only one as the structure and humus content of the soil likewise play an important part. The protozoa found were all flagellates.

Another experiment with three different soils under different moisture conditions, part sterilized and part unsterilized, the latter plus dried blood, to each of which cultures of protozoa were added, brings out the fact that the sterilization of soil and addition of easily soluble organic matter will make the conditions optimum for protozoan activities at a lower moisture content than in corresponding unsterilized or untreated soils.

Protozoa (flagellates) found in the first inch of three cultivated soils varied in numbers from 1000 to 10,000 per gram of soil; at four inches, from 100 to 5000; at eight inches from 100 to 5000; at twelve inches from 10 to 100. None were found at a depth of twenty or thirty inches. The fourth soil, an acid forest soil, contained from 10 to 100 flagellates at a depth of one inch and at four inches; at eight inches only 1 to 10 were found and below that none. Ciliates and amoebae were not recorded as they occurred only occasionally.

Flagellates are present in greatest numbers at a depth of 1 inch where conditions favor microörganic activities, ciliates and large flagellates in largest numbers at a depth of 4 inches where the moisture conditions may be more favorable for their development. A list of the common types is given.

The flagellates are at once the most common and the largest group of soil protozoa.

Ammonification by bacteria is not influenced appreciably by soil protozoa although their presence acts detrimentally upon bacterial numbers.

This might be explained by one of the following assumptions: (1) if the protozoa destroy bacteria, they destroy non-ammonifying organisms; 2) the protozoa themselves take part in the process of ammonification; (3) the disintegration of the bacterial cells results in decomposition products which might be responsible for high ammonia production.—Z. N.

A Detailed Study of Effects of Climate on Important Properties of Soils.
C. B. LIPMAN and D. D. WAYNICK. (Soil Science, 1916, 1, 5–48, 5
plates.)

A soil block, 5 feet square and 3 feet deep from the fields of each of
three state experiment stations (Maryland, Kansas and California) was
moved to the two other experiment stations and placed in position as
nearly as possible in the original order of layers. A similar block of soil
was dug up and replaced in its position at every station. Strips 5 feet
wide of untouched field soil surrounded the board frame of every plot
which was placed in position. It was then possible to study at Mary-
land, at Kansas and at California four soil blocks as follows: First, nat-
ural field soil *undisturbed;* second, natural field soil *disturbed* and re-
placed; third and fourth, soil blocks obtained respectively from each of
the other two stations. The chief aim was to show how any given soil
in its natural location compares, after seven years, with the same soil
under foreign conditions. Unfortunately no studies except brief chemi-
cal studies, were made at the initiation of the soil exchange experiment.

Studies of the hygroscopic coefficient, the moisture equivalent, the
wilting point, changes in color and colloidal nature were among the
physical studies made. Among chemical studies, complete chemical
analyses were made in accordance with the official method, also humus
and humus nitrogen determinations, total nitrogen and soil water-ex-
tract studies. Among bacteriological studies were: Counts on albu-
men agar, ammonifying power for dried blood, nitrifying power for the
soil's own nitrogen, for dried blood, for cotton-seed meal, and for sul-
fate of ammonia, nitrogen fixing power in mannit solution, and qual-
itative tests for cellulose destruction.

The soils were described as follows: The California soil as "Sacra-
mento silt loam," the Kansas soil as a "dark heavy loam," and the Mary-
land soil as a "light yellow clay."

It was found that soils change markedly in color in a period of seven
years, and perhaps less, when moved to other climates. The differ-
ences are so great that samples of any one original soil from the three
different stations today show no outward resemblance among them-
selves, but appear to represent three very distinct soil types.

In general the hygroscopic coefficient, the moisture equivalent, and
the wilting point of any of the soils increased when the soil was placed
at California. Some exceptions to this rule are noted.

Generally speaking bacterial numbers increase in arid soils placed
under humid conditions. In general, also, the opposite is true for
humid soils.

Ammonification, nitrification and nitrogen fixation follow the general
trend of bacterial counts. In the case of nitrification, however, this
applies to certain forms of nitrogen only. In the case of other forms
of nitrogen very peculiar conditions exist which are fully explained in
the text.

Cellulose destruction by soils proceeds with greater rapidity under
arid than under humid conditions with any given soil type. Cellulose

destruction therefore appears to follow in general an opposite course to those of other microörganic activities in soils as affected by climate.

Marked changes in the acid soluble constituents of soils are wrought by climatic effects. The general tendency is for soils to increase in iron and decrease in alumina when placed under arid conditions, and vice versa.

Phenomenal losses in certain constituents in five years seem to have occurred in some soils even when the latter were not moved. Thus for example the Maryland soil lost in the period named enormous quantities of magnesia.

Large increases occur in the total water soluble constituents of California soil when it is moved to the Kansas or Maryland stations. On the other hand, the Maryland soil gains in water soluble matter when moved to Kansas or to California.—Z. N.

BACTERIOLOGY OF THE MOUTH

The Treatment of Pyorrhea Alveolaris and its Secondary Systemic Infections by Deep Muscular Injections of Mercruy. B. D. WRIGHT. (Medical Record, 1916, **89**, 807–811.)

Seventy-five cases of pyorrhea were treated with mercuric succinimide administered intramuscularly. In. conjunction with the injections of mercury, surgical treatment of the gums and teeth was carried out.

In all cases, the pyorrhea was entirely cured. Secondary systemic infections, which were present in 54 per cent of the patients, were also cured in every case.

The author is convinced that mercury, because of its parasitotropic action, promises to be successful in treating various infections produced by the vegetable parasites.—M. W. C.

Constitutional Conditions Caused by Oral Sepsis. J. DALAND. (New York Med. Jour., 1916, **103**, 817–820.)

Foci of infection in the mouth may give rise to serious constitutional disturbances, such as endocarditis, acute parenchymatous nephritis, furunculosis, and particularly septic polyarthritis. Many other pathologic conditions may be traced to a septic mouth as the cause. The offending organism, in such cases, is usually a streptococcus. This bacterium, according to the work of Rosenow, may develop greater virulence than it possesses as it exists in the mouth, if it is transferred to other parts of the body where different conditions for growth are present.

As a direct causal relationship exists between oral sepsis and constitutional disease, the dentist should take particular pains to remove all manifestations of oral sepsis, and it should become a matter of routine procedure that any septic conditions of a patient's mouth should be reported to the physician.—M. W. C.

A Study of Endameba Buccalis in Alveolodental Pyorrhoea. F. M. JOHNS. (Am. Jour. Trop. Dis. and Prevent. Med., 1916, 7, 372–376.)

The first part of this paper deals with a microscopic study of the lesion—demonstrating the presence of the *Endameba buccalis* in greatest numbers penetrating the healthy peridental tissue beyond the superficial zone of suppuration, and not living in the mass of bacteria, pus and semi-necrotic material filling the pocket.

Phagocytosis of bacteria is only noted in the few atypical forms that are swept into the pus of the pocket. From the depths of the lesion only a large nucleated cell is found to be constantly ingested.

Reproduction in the lesion is apparently only by bilateral fission. Cyst formation was not observed.

A significant fact was in the adhesion of numbers of bacteria to the ameba when observed in fresh and wet fixed preparations.

Endamebae following the injection of emetin are gradually decreased in numbers without any visible morphological or biological changes being produced. Forms in active reproduction are found up to the complete disappearance, the inference being drawn that the action of emetin upon pathogenic amebae is an indirect one in the dosage clinically possible.—F. M. J.

BACTERIOLOGY OF WATER AND SEWAGE

Bacterial Counts in Water Examination. J. RACE. (Amer. Jour. of Pub. Health., 1916, 6, 488–496.)

The conclusions as given by the author are:

1. Not one of the media used at any one of the incubation periods or temperatures chosen gives a bacterial count that bears a constant ratio to the organisms of excremental origin as estimated by the *B. coli* test.

2. The blood heat count is the nearest to the *B. coli* content.

3. The largest count is obtained by the use of nutrient gelatin.

4. The bacterial count varies directly with the incubation period and inversely with the incubation temperature.

5. The largest count in the shortest incubation period is obtained at 27°C.

6. The ratio of the count at a given temperature to the count at a higher temperature decreases as the temperature of the water increases.

D. G.

Bacteria in Commercial Bottled Waters. MAUD MASON OBST. (Bulletin No. 369, U. S. Dept. of Agr.)

The paper is based on results obtained from the examinations in the Bureau of Chemistry during the last six years of from 1 to 17 samples of bottled waters from each of 110 American springs and from 57 sources in foreign countries.

The author concludes that the data as summarized show the need of improvement in the bacteriological condition of many of the brands of bottled water to be found in the market.   There are some springs used for the production of commercial bottled waters which should not be used.   In other cases, the contaminations found are clearly those of manipulation.

The results clearly show that bottled water can be made to conform to the requirements of the United States Public Health Service for drinking water furnished upon trains; that is, that not more than one 10 cc. sample out of five should show the presence of *B. coli*.—S. H. A.

Confirmatory Tests for B. coli in Routine Water Examinations.   W. H. FROST.   (Amer. Jour. of Pub. Health, 1916, **6**, 585–588.)

If no typical colonies develop within 24 hours on Endo plates made from fermentation tubes showing gas, further effort is made to recover *B. coli* as follows: (1) One or more colonies are transferred to lactose broth fermentation tubes.   The formation of gas demonstrates presence of *B. coli*.   (2) Plates are again made from the original fermentation tube.   (3) A transplant is made from the original fermentation tube directly to another lactose broth tube.   If steps (1) and (2) have both failed to recover *B. coli*, plates are are now made from this transplanted culture.   (4) At the same time a transfer is made from this tube directly to a third fermentation tube.

If all of the above procedures fail to recover *B. coli* and gas is still formed in this fermentation tube, the inference is that gas in the preliminary test was due to an anaerobe.

The presumptive test for *B. coli* whether in lactose bile or lactose broth is subject to considerable variations in its specificity as indicating the demonstrable presence of *B. coli*.   Careful confirmation of the presumptive test is especially important in the examination of treated waters, where the error in this test is greater than in raw waters.—D. G.

CLASSIFICATION OF BACTERIA

Studies on the Classification of the Colon-Typhoid Group.   J. B. THOMAS and E. A. SANDMAN.   (Amer. Jour. of Pub. Health, 1916, **6**, 579–584.)

The conclusions as given by the authors are: Glycerine is of value in separating *B. cloacae* from *B. communior* and *B. aerogenes*, but is fermented by both of the latter types, while *B. communis* isolated from water frequently fails to ferment it.

Dulcite and adonite are of little value in separating *B. cloacae* from *B. aerogenes* and *B. communior* while a majority of *B. communis* isolated from water fail to ferment these substances.

Salicin is frequently fermented by all the lactose-positive organisms.

Raffinose is frequently fermented and dextrin usually fermented by all of the lactose-positive organisms.—D. G.

A Study on the Grouping of Meningococcus Strains. MIRIAM P. OLM-
STEAD, PHOEBE L. DUBOIS, JOSEPHINE B. NEAL, and ROSE SCHWEIT-
ZER. (Journal of Immunology, 1916, **1**, 307.)

Twenty-nine strains of meningococci were studied as to their im-
munity reactions by the complement fixation method. Immune sera
were prepared in rabbits with each of these strains, and cross fixations
were carried out with extracts of each strain of cocci. Fourteen strains
gave cross fixation and fell into one group. Eight other strains gave
cross fixation and so fell into a second group. Three strains gave cross
fixation with certain members of group 1 but not with all, and two
strains were entirely heterogeneous. The organisms of the second group
gave cross fixation with one strain of Dopter's parameningococcus but
not with a second representative of his organisms.—J. G. H.

*The Correlation of the Voges-Proskauer and Methyl Red Reaction in the
Coli-Aerogenes Group of Bacteria.* MAX LEVINE. (Jour. Infect.
Diseases, 1916, **18**, 358–367.)

The author studied the reactions of 167 colon-like organisms from
the horse, cow, pig, sheep, man, raw sewage and septic sewage. It was
shown that the two reactions studied were correlated; further that (1)
there was a better correlation between saccharose fermentation and
source than between saccharose-dulcite fermentation and source; (2)
cultures which formed acetyl-methyl-carbinol from glucose usually fer-
mented salicin and glycerin, but dulcite only occasionally; (3) organisms
that give the Voges-Proskauer reaction are rarely found in feces; (4) in
this test the glucose-peptone-dipotassium phosphate medium of Clark
and Lubs gives a more distinct test than glucose broth. Since this re-
action is characteristic for non-fecal strains, the author concludes that
it may be of considerable sanitary significance.—P. B. H.

DAIRY BACTERIOLOGY

Pasteurization as a Public Health Measure. C. M. HILLIARD. (Jour.
Home Economics, 1916, **8**, 307–312.)

The article gives a comprehensive statement of the principles and
methods of milk pasteurization, and discusses the relation of the prac-
tice to the public health, especially the health of infants. Bacterial
reductions obtained, both quantitative and qualitative, are consid-
ered.—C. M. H.

Bacterial Testing Versus Dairy Inspection. C. E. NORTH. (Amer.
Jour. of Pub. Health, 1916, **6**, 569–578.)

The author discusses the value of intelligent coöperation of labora-
tory worker and inspector. The function of the laboratory is to exam-
ine results and to locate insanitary milk. The inspector's function is
to ascertain the causes and to apply the remedies. Simple laboratory
equipment and methods within the reach of the small village are dis-
cussed.—D. G.

Some Observations on Causes of High Bacterial Counts in Milk. H. D. PEASE. (Amer. Jour. of Pub. Health, 1916, **6**, 563–568.)

In parts of the country where adequate field and laboratory investigations of milk supplies have been in operation high bacterial counts are most generally caused through inefficiently cleaned apparatus, or as a result of inefficient refrigeration. In locations where no supervision has been in operation and where the producers are more or less slovenly, high bacterial counts may be attributed to definite dirty conditions and are found even where the milk can be shown to have been delivered promptly and with the application of a reasonable degree of refrigeration.—D. G.

Colon Bacteria and Streptococci and Their Significance in Milk. L. A. ROGERS, W. M. CLARK, and A. C. EVANS. (Amer. Jour. Public Health, 1916, **6**, 374–380.)

The authors find that cultures (streptococci) isolated from infected udders are characterized by weak fermentation of the simpler sugars and an inability to ferment higher polysaccharides and alcohols, while cultures from bovine feces may be distinguished by ability to ferment polysaccharides and inability to attack alcohols. They believe that the presence in lactose bile inoculated with milk and incubated at 37°C. of streptococci forming distinct chains is good presumptive evidence of milk from infected udders.

Bacteria of the colon group occurring in market milk may be divided into two very distinct groups (1) that which agrees closely with the characteristic colon bacillus of the bovine intestine, yielding a low $CO_2: H_2$ ratio; (2) that which yields a high ratio; numerous in milk, but occurring very rarely in bovine intestine (1 out of 150 cultures).

The surface of dried grains is found to be source of a number of types of colon. That type occurring with greatest frequency probably corresponds to conception of *B. lactis aerogenes,* so that the ordinary presumptive tests and even the usual confirmatory tests are not necessarily proof of the contamination of the milk with fecal matter.—D. G.

DISINFECTION

The Antiseptic Action of Ether in Peritoneal Infections. J. SALIBA. (Jour. A. M. A., 1916, **66**, 1295–1297.)

The instillation of ether into the peritoneal cavity in infections by streptococci and *B. coli* served as a safe and beneficial antiseptic.—G. H. S.

The Bactericidal and Fungicidal Action of Copper Salts. L. M. DeWITT and HOPE SHERMAN. (Jour. Infect. Diseases, 1916, **18**, 368–382.)

The present study was initiated to ascertain (1) the killing power of the sulphate and chloride of copper and (2) their inhibiting action, on *B. coli, B. typhi, B. prodigiosus, B. tuberculosis* and *Staph. aureus;* also on species of yeast, *Aspergillus* and *Penicillium.* The results indi-

cated that copper is unreliable as a bactericide and as a fungicide, although some organisms are more susceptible than others. Long time experiments were more satisfactory than short time tests. It is said that solutions representing one part of the metal (2.5 parts of the chloride or 4 parts of the sulfate) to the million kill the ordinary water organisms *B. coli* and *B. typhi*, but do not injure the health. One part to 100,000 was found to inhibit the growth of *B. tuberculosis* in vitro. The therapeutic value of copper salts is discussed.—P. G. H.

IMMUNOLOGY

Immunity, Natural and Acquired. W. H. PORTER. (Medical Record, 1916, **89**, 983–987.)

A theory of immunity which bases the formation and activities of antibodies upon the metabolic processes of the body proteins.—M. W. C.

The Production of a Hyperimmune Serum for Infectious Abortion in Mares. E. S. GOOD and WALLACE V. SMITH. (Jour. Infectious Diseases, 1916, **18**, 347–401.)

The authors report on the production of a serum which protected rabbits from the lethal dose, but did not protect a mare from artificial infection. In a guinea pig the time for abortion was lengthened.—P. B. H.

Complement Fixation in Vaccinia and Variola. JOHN A. KOLMER. (Journal of Immunology, 1916, **1**, 59.)

The sera of vaccinated rabbits and of a few recently vaccinated human beings gave complement fixation with salt solution extracts of variola virus and vaccine virus. Positive reactions were also obtained in 9 out of 17 smallpox patients. Alcoholic extracts of the virus could not be used as antigens as they gave no reactions with these sera.—J. G. H.

Complement Fixation in Varicella. JOHN A. KOLMER. (Journal of Immunology, 1916, **1**, 51.)

Kolmer obtained weak complement fixations in 7 out of 24 cases of chicken pox, the antigen used being a saline solution of the contents of the varicella vesicles.—J. G. H.

The Agglutinability of Blood and Agar Strains of Typhoid Bacilli. C. G. BULL and I. W. PRITCHETT. (Jour. Exp. Med., 1916, **24**, 35–40.)

Cultivation on 10 per cent rabbit blood agar did not affect the agglutinability of fifty-seven strains of typhoid bacilli. The authors were unable to confirm the observations of Gay and Claypole on the variation in agglutinability caused by cultivating the typhoid bacillus on blood agar. A typhoid bacillus showing irregularity in fermentation, agglutination, and indol production is described.—B. W.

A Simplified Method of Producing a Potent Precipitin Serum. WALLACE
V. SMITH. (Jour. Med. Res., 1916, **34**. 169–175.)

The author finds that the serum proteins precipitated from blood
by means of one-third saturation with ammonium sulphate consti-
tute a potent antigen for the preparation of precipitin serum in rab-
bits. The dried precipitate can be kept on hand over a long period
without impairment.—H. W. L.

*The Agglutination Reaction with Sera Derived from Human Cases of
Leprosy and from the Experimental Animal upon Various Members of
the Acid-Fast Group.* W. H. HARRIS and J. A. LANFORD. (Jour.
Med. Res., 1916, **34**, 157–167.)

Sera from 20 human cases of leprosy tested against several strains
of *B. leprae*, human, bovine, and avian tubercle bacilli, and several of
the non-pathogenic acid-fasts, failed to show any regularity or speci-
ficity. The same was true of experimental sera produced by injections
of the various strains of leprosy bacilli into rabbits.—H. W. L.

Observations on the Typhoid Reaction. C. R. AUSTRIAN and A. L.
BLOOMFIELD. (Archives of Internal Medicine, 1916, **17**, 663–669.)

By the typhoidin reaction individuals who had suffered from typhoid
fever or received prophylactic treatment could not be differentiated
from those who had never had the disease nor received vaccine treat-
ment. The reaction was positive in a large percentage of persons who
gave no history of typhoid fever or artificial immunization.—G. H. R.

*Erysipelas Migrans and Multiple Abscesses in a Six Months' Old Infant
Successfully Treated with Vaccines.* L. FISCHER. (Medical Record,
1916, **89**, 734–735.)

Report of a case, which showed no response to treatment with leuco-
descent light, ichthyol, evaporating lotions, or magnesium sulphate, but
in which decided improvement and finally complete recovery followed
the administration of stock streptococcus and autogenous vaccines.—
M. W. C.

Vaccine Treatment. LUDVIG HEKTOEN. (Jour. A. M. A., 1916, **66**,
1591–1594.)

A discussion of the theory and practise of vaccine therapy. Follow-
ing accurate diagnostic procedure an autogenous vaccine would seem
the logical method of treatment. Stock vaccines and phylacogens,
which are not standardized and may not be specific, have a limited
application.—G. H. S.

*Is the Hyperleucocytosis Following the Injection of Typhoid Bacilli into
Immunized Rabbits Specific?* HELEN I. McWILLIAMS. (Journal of
Immunology, 1916, **1**, 159.)

The experiments of Gay and Claypole showing hyperleucocytosis in
immune rabbits following the injection of typhoid bacilli, were reviewed,

with the special object of determining whether this reaction was specific. The leucocytosis which McWilliams obtained in immune rabbits was no greater than that which normal rabbits showed and the typhoid immune rabbits reacted as strongly to colon as to typhoid bacilli.—J. G. H.

Specific Therapy in Certain Acute Infectious Diseases. F. J. DEVER. (New York Med. Jour., 1916, **103**, 972–975.)

Treatment of infectious diseases by their specific immune sera holds more promise of success than an active immunization with either plain or sensitised vaccines. This is due to the fact that the active immunity obtained by vaccines is produced relatively slowly and is of questionable value in an acute infection, while a specific serum, on the other hand, supplies antibodies without putting any added strain upon the body cells.—M. W. C.

Intravenous Serobacterin Therapeutics. W. E. ROBERTSON. (New York Med. Journ., 1916, **103**, 777–780.)

In acute infectious diseases, such as typhoid fever and pneumonia serobacterins, when administered intravenously, shorten the incubation period, and produce a particularly striking change in the blood picture, the most noticeable feature of which is a pronounced leucocytosis.

In local and chronic diseases the value of the intravenous use of serobacterins, as compared with other bacterins, has not as yet been tested.—M. W. C.

The Value of Autoserum Injections in Skin Diseases. W. S. GOTTHEIL. (New York Med. Jour., 1916, **103**, 1209–1211.)

Autoserum treatment in skin diseases does not itself effect a cure, but used in conjunction with local treatment it shortens the period of disease from weeks to days and lessens the probability of relapse.

Autoserum treatment is particularly useful in psoriasis. It is of frequent benefit in chronic urticaria, neurodermatitis, pruritus senilis and other itchy dermatoses; of some value in pustular acne and chronic eczema; and of no use in furunculosis, folliculitis, and other pus infections as well as pemphigus, lepra, lichen planus and syphilis.—M. W. C.

Autosensitized Vaccines. M. G. WOHL. (Medical Record, 1916, **89**, 770–772.)

Serobacterins which are sensitised with the patient's serum are more efficient than vaccines sensitised with a heterologous serum. This is due to the fact that the antibodies in the sera of the lower animals are not identical with those of human serum, and therefore cannot act as effectively as those found in the patient's serum.

An additional reason why autosensitised vaccines are superior is that the bacteria do not need to be freed from the immune serum by washing, but may be injected with the serum used in sensitising.—M. W. C.

Gonococcus-Complement Fixation: A New Lipoid Antigen. C. C. WARDEN and L. E. SCHMIDT. (Jour. of Lab. and Clin. Med., 1916, **1,** 333–347.)

In complement fixation tests for gonorrhea, an antigen composed of an alcoholic solution of the fats of the gonococcus gave a much higher percent of positive reactions than a commercial watery antigen (Parke, Davis and Company). With the Warden antigen the positive reactions appeared earlier, persisted longer, and occurred in a larger number of doubtful cases. Every case which gave a positive reaction with commercial antigen was positive with the Warden antigen and usually to a much greater degree.—M. W. C.

The Bio-Chemistry of the Gonococcus in its Relation to Immunity. CARL C. WARDEN. (The Urologic and Cutaneous Review, 1916, **20,** 181– 182.)

On analysis Gonococcus substance shows 12 per cent nitrogen, 20 per cent fat, together with phosphorus, sulphur, salts and ash. Washing removes nitrogen and fats. Gonococci possess at least three enzymes, one proteolytic, one hydrolytic and one a lipase.

An aqueous or normal salt suspension of gonococci behaves, physically, as a colloid with an electronegative sign and is flocculated or agglutinated by electrolytes of opposite sign and by other colloids such as serum, under certain conditions.—C. P. B.

The Complement Deviation Reaction Applied to the Diagnosis of Moniliasis of the Digestive Tract. I. G. MARTINEZ. (Am. Jour. Trop. Dis. and Prevent. Med., 1916, **7,** 390–391.)

An extract of 10 cc. normal saline extract of three agar tubes of different strains of monilias was made from seventy-two hours growth on Sabourand's glucose agar. These were shaken for two hours, 0.5 per cent carbolic acid added, and the extract heated for one hour at 56°C. This antigen was titrated according to the usual method, and 0.4 cc. found to constitute one unit.

In use, following the original Wassermann technic for complement deviation reaction, two cases of sprue and one suspected case gave a positive reaction. All other reactions with other sera were negative. —F. M. J.

Variations in the Pneumococcus Induced by Growth in Immune Serum. L. M. STRYKER. (Jour. Exp. Med., 1916, **24,** 49–68.)

Various strains of highly virulent pneumococci of Types I and II were grown and subcultured for successive generations in media containing homologous immune serums. The author found that the serum treated pneumococci became less specifically agglutinable, they could no longer absorb the agglutinins for normal strains, and, when injected into the animal body, failed to produce agglutinating sera for normal strains. Further, the virulence of the various treated strains was decreased, which may be due to the absence of capsules on such strains and

also to the fact that the organisms so grown are phagocyted in normal serum.   It is interesting to note that the variations in pneumococci produced by treatment with immune serum do not persist after animal passage.   Reversion to the normal type takes place readily.—B. W.

Further Observations on the Agglutination of Bacteria in Vivo.   C. G.
    BULL.   (Jour. Exp. Med., 1916, 24, 25–34.)
    Pneumococci, dysentery bacilli of the Shiga type, and *Bacillus muco-sus-capsulatus* are agglutinated immediately when injected into the circulation of actively immunized rabbits.   *Staphylococcus aureus* and *albus*, colon bacilli, meningococci, gonococci, and non-virulent pneumo-cocci agglutinate in the circulation of normal rabbits.   Bouillon cultures of *Bacillus avisepticus* are highly toxic for both rabbits and dogs. The fresh sera of these animals have no bactericidal action upon the bacteria.   Dog serum opsonizes the bacilli *in vitro*, and they are agglutinated and opsonized in the circulation and organs of normal dogs.   On the other hand, this does not occur in connection with normal rabbits. A very small quantity of culture produces a fatal septicemia in rabbits but a subtoxic dose is without effect in dogs.   The degree of agglutination and opsonization of bacteria within the animal body is inversely parallel to the infectiousness of the bacteria for the host.—B. W.

A Simple Method of Quantitative Determination of Complement Fixation.   J. O. HIRSCHFELDER.   (Jour. A. M. A., 1916, 66, 1386–1387.)
    The author gives his technic for performing complement fixation tests.—G. H. W.

The "Delayed Negative" Wassermann Reaction.   O. M. OLSON.   (Jour.
    Lab. and Clin. Ned., 1916, 1, 704–705.)
    The "delayed negative" reaction differs from the original Wassermann only in the method of reading the test.   Instead of one reading at the end of two hours, readings are taken at twenty minute intervals while the tubes are in the incubator.
    By this method the progress of hemolysis may be observed, and the relation of the time of reaction between the positive and negative controls and the serum under test may be noted.
    Whenever hemolysis begins later and is slower in producing complete lysis than the negative control, the serum under test is called a "delayed negative" and is considered indicative of syphilis.—M. W. C.

The Antagonistic Action of Negative Sera upon the Wasserman Reaction.
    A. W. SELLARDS and G. R. MINOT.   (Jour. Med. Res., 1916, 34, 131–147.)
    The authors report observations on the ability of normal negative sera to antagonize the reaction between a positive syphilitic serum and antigen.   Working on the theory that, with a non-specific antigen such as is now commonly used for the Wassermann, binding may result from either an increase of specific complement-fixing antibodies, or a de-

crease in the normal antagonistic action, they were able to show that negative sera possessed, in varying degrees, substances which inhibited the binding power of positive sera. Of 119 cases tested, only six failed to show this antagonistic action. All negative sera were inactivated and none were used which contained two units or more of natural sheep hemolysin. Although of no practical application in routine Wassermanns, this factor may be of importance in complement fixation in other diseases.—H. W. L.

Further Observations on the Schick Test for Diphtheria Immunity. G. B. WEAVER and B. RAPPAPORT. (Jour. A. M. A., 1916, **66**, 1448–1450.)

General discussion of the subject with data upon the presence of the reaction in healthy adults, in cases of post diphtheritic paralysis, in scarlet fever patients, and in tonsillitis patients.

The authors employ toxin neutralized to excess with antitoxin as a control.—G. H. S.

The Schick Test, D. M. GRISWOLD. (Jour. Lab. and Clin. Med., 1916, **1**, 441–443.)

Beside providing a quick and easy method for determining individual susceptibility to diphtheria, the Schick test affords a means of determining the duration of the passive immunity conferred by injections of antitoxin. Of six convalescents tested every second day after they had recovered from diphtheria, the earliest recurrence of a positive Schick was 3 weeks after 20,000 units of antitoxin, the longest period of immunity, six weeks.—M. W. C.

The Pseudoreaction in the Schick Test and its Control. A. ZINGHER. (Jour. A. M. A., 1916, **66**, 1617–1618.)

The Schick test is due to the action of the soluble diphtheria toxin while the pseudoreaction is an anaphylactic response to the protein of the diphtheria bacillus.

To control such pseudoreactions an injection of diphtheria toxin heated to 75°C. for five minutes may be given. The soluble toxin is thus destroyed and any reaction following such an injection is due to the protein of the diphtheria bacillus.—G. H. S.

The Diphtheria Toxin Skin Reaction. H. KOPLIK and L. J. UNGER. (Jour. A. M. A., 1916, **66**, 1195–1196.)

A simplified method of performing the Schick test is described.

Instead of the diluted toxin, with the necessary sterile accessories, pipettes, syringes, etc. the authors employ undiluted toxin and an ordinary hypodermic needle.

The needle is dipped into the toxin and introduced intradermally. The advantages of this method, aside from its simplicity, are that undiluted toxin will retain its potency, traumatic pseudopositive reac-

tions are entirely eliminated, and anaphylactic pseudopositive reactions are largely eliminated.

As controlled by the Schick technic complete uniformity resulted, showing that the amount of toxin introduced was sufficiently accurate to secure reliable results.—G. H. S.

Diphtheria Immunity—Natural, Active and Passive. Its Determination by the Schick Test. W. H. PARK and A. ZINGHER. (Amer. Jour. of Pub. Health, 1916, 6, 431–445.)

The test is of great reliability when properly made. In a certain proportion of individuals a pseudo-reaction is seen but this can usually be distinguished clinically from the true reaction. The test possesses great value in determining clinically the immunization of susceptible individuals, and in clearing up the diagnosis of clinically doubtful cases of diphtheria. The Schick reaction has added further proof to the clinical and experimental observations that very toxic cases of diphtheria do better when given an early intravenous injection of antitoxin than when it is administered in any other way. The results obtained with the test in families seem to indicate that besides infection with virulent diphtheria bacilli, other factors, possibly hereditary in nature are concerned in the production of natural immunity to diphtheria. The Schick reaction can be applied with advantage in testing the patients, resident staff and nurses of contagious disease hospitals. By its use a considerable saving can be effected in antitoxin during diphtheria outbreaks. A more widespread use of an active immunization with mixtures of diphtheria toxin and antitoxin would help to lessen the disease.—D. G.

Immunity in Tuberculosis. G. A. WEBB. (Jour. of Laboratory and Clinical Medicine, 1916, 1, 414–427.)

A comprehensive résumé of work upon immunity in tuberculosis.

Attempts of the author to immunize guinea-pigs with living tubercle bacilli were successful when gradually increasing numbers of individuals of an old human culture were used, but unsuccessful with recently isolated cultures.

Experiments with monkeys inoculated with gradually increasing doses of a human culture produced in some animals a resistance to 10,000 times the lethal dose. Such a resistance could not be constantly produced, however. Lack of success with monkeys was probably due to excessive virulence of the culture.

Children inoculated five years ago with a culture of which the minimal lethal dose for a guinea-pig was 125 bacilli are still healthy and do not respond to the von Pirquet test.

The author is convinced that infection takes place, usually in childhood, with very small numbers of bacilli. If an immunity in childhood could be raised to even a slight degree, it would probably be possible to vaccinate successfully against tuberculosis.—M. W. C.

Immunity Factors in Pneumococcus Infection in the Dog. C. G. Bull. (Jour. Exp. Med., 1916, **24**, 7–24.)

Intravenous inoculations of from 1 to 3 cc. per kilo of body weight of a bouillon culture of virulent pneumococci produce septicemia and meningitis in dogs. The injected penumococci leave the circulation rapidly, but begin to reinvade the blood from twenty-four to forty-eight hours later. The septicemia reaches its climax between the fourth and fifth days and then abruptly declines, the blood becoming sterile within from one to three days after the height of the septicemia is reached. The initial disappearance of the pneumococci from the circulation has been found to be due to agglutination of the diplococci in the blood stream and accumulation of the clumps in the lungs, liver, spleen, etc. If the dogs are reinoculated during the ascension of the septicemia, the injected diplococci leave the circulation as rapidly as in normal dogs. Cultures isolated in this stage of the infection, both before and from three to four hours after the reinoculation, are resistant to the agglutinins and opsonins of immune sera that agglutinate and opsonize the cultures with which the dogs were originally infected. Thus it follows that the pneumococci are able to reinvade the circulation because they have acquired a fastness to the existing antibodies and not because the antibodies have been exhausted. By reinoculating dogs at the time of the crisis in the septicemia it has been shown that the agglutination of the pneumococci is more rapid and complete and that the diplococci leave the circulation much more rapidly than in normal dogs. Hence acquired antibodies are operative within the animals at this time although they cannot be demonstrated *in vitro* until from twenty-four to forty-eight hours later. Pneumococci isolated as the infection is subsiding are more susceptible to the action of immune sera than the original cultures injected. It is probable that all the dogs would have survived the infection if a meningitis had not developed. In the acutely fatal cases of meningitis few pneumococci are phagocyted, while in the milder and convalescent cases much phagocytosis occurs. It is suggested that the incubation period of infectious diseases is due to the fact that the infecting agents must become adapted to the adverse conditions encountered in the newly infected host before they can multiply sufficiently to produce the symptoms of disease. It is further suggested that epidemics may arise because the infectious agent is passed from person to person in the ascending stage of the disease and thus enters new hosts in a state of maximum resistance to the natural antibodies of such individuals. When early contacts are avoided, epidemics tend to subside because the infectious agent is weakened by the action of acquired antibodies during the period of convalescence.—B. W.

LABORATORY TECHNIQUE

Hydrochloric Acid as a Decolorizing Agent for the Tubercle Bacillus. R. A. KEILTY. (Jour. A. M. A., 1916, **66**, 1619–1620.)

The following technic is given: Make thin smears, fix with heat, and stain with cold carbolfuchsin for five minutes. Decolorize for thirty seconds or more with 30 per cent hydrochloric acid. Counterstain with Loeffler's methylene blue.—G. H. S.

A Device for Protection against the Tubercle Bacillus. R. A. KEILTY. (New York Med. Jour., 1916, **103**, 1074.)

The device described is used to prevent the scattering of tubercle bacilli from a platinum loop while it is being flamed.

The apparatus consists of a tube of Russian iron, so constructed that it surrounds the flame of a Bunsen burner, fitting the burner tightly at the bottom. The platinum loop may be plunged into the flame and any masses which jump are caught upon the sides or bottom of the tube.—M. W. C.

Stabilized Gentian Violet. W. D. STOVALL and M. S. NICHOLS. (Jour. A. M. A., 1916, **66**, 1620–1621.)

To prevent deterioration of the gentian violet used in Gram's stain the authors suggest a stain of the formula:

Anilin	28 cc.
Gentian violet	8 gm.
95 per cent alcohol	100 cc.
N Hydrochloric acid	5 cc.
Distilled water	qs. ad 1000 cc.

Dissolve gentian violet in the alcohol. Add hydrochloric acid to the anilin and dissolve in water to make 900 cc. Filter the aqueous solution and add to the alcoholic stain. Filter.—G. H. S.

The Production and Collection of B. coli in Quantity on Synthetic media. ROBERT BENGIS. (Jour. Infect. Diseases, 1916, **18**, 391–396.)

The best medium was composed of the following: 2.5 per cent agar, 1 per cent ammonium lactate, and 0.2 per cent disodium phosphate, with or without 1 per cent calcium carbonate. The absence of lactose increased the efficiency. Inoculation of specially constructed plates was accomplished by means of a De Vilbiss atomizer. The growth was scraped off with a safety blade and placed in 75 per cent cent alcohol.—P. B. H.

Production of Clear and Sterilized Anti-Hog-Cholera Serum. M. DORSET and R. R. HENRY. (J. Agr. Res., 1916, **6**, 333–338.)

The occasional presence of the foot-and-mouth virus in hog-cholera serum makes it necessary to devise some means of sterilizing it. Heating for thirty minutes at 60° kills the foot-and-mouth virus; but unless

the corpuscles are removed from the defibrinated hog-cholera-immune blood, the heat causes coagulation and destroys the commercial value of the serum. Complete removal of the corpuscles by centrifugalization alone has proved impractical; but if they are first agglutinated by the addition of extract of the common white navy bean, complete separation from the serum is quite easy. The serum thus prepared has no harmful properties due to the bean extract; and can be heated for thirty minutes at 60° without undergoing any change.—H. J. C.

PLANT PATHOLOGY

Crown gall Studies; Showing Changes in Plant Structure Due to a Changed Stimulus. (Preliminary paper.) ERWIN F. SMITH. (J. Agr. Res., 1916, **6**, 179–182, with six plates.)

This is a continuation of work on crown gall in which its similarity to human cancer is pointed out. This paper describes some new points in the pathology of the disease.—H. J. C.

Transmission and Control of Bacterial Wilt of Cucurbits. F. V. RAND and ELLA M. A. ENLOWS. (J. Agr. Res., 1916, **6**, 417–434.)

It was shown several years ago by Erwin Smith that this disease (due to *B. tracheiphilus*) was transmitted by the striped cucumber beetle (*Diabrotica vittata* Fab.). The present work confirms this conclusion, and shows that the principal summer carriers of the disease are all species of Diabrotica. There is no evidence of spread through the soil (unless the roots are injured) or by means of infected seed. The disease can be controlled by early treatment with Bordeaux and arsenate of lead. Experiments are now being carried on to learn how to control the beetles that spread the disease.—H. J. C.

Further Evidence that Crown Gall of Plants is Cancer. E. F. SMITH. (Science, 1916, **43**, 871–889.)

After a brief survey of the main sub-divisions of the forms of cancer and their outstanding characteristics, the author presents a powerful argument for the "parasitic origin and essential unity of the various forms of cancer occurring in men and animals." The evidence is essentially presumptive, being drawn from the striking resemblance between Crown Gall of plants, which the author has proved to be caused by the *Bacterium tumefaciens*, and human cancer. The domination of the morphologists in cancer research is deplored and the attention of the experimental biologist and the bacteriologist is invited. So firm is the author's conviction of the parasitic nature of this malady that he exclaims: "I am now persuaded that the solution of the whole cancer problem lies in a study of these plant tumors." The discovery reported that the Crown Gall organism will produce teratoid tumors with regularity in various plants, not only in dormant buds but in the leaves, is a most astonishing and important contribution.—C. M. H.

Studies on the Crown Gall of Plants: Its Relation to Human Cancer.
ERWIN F. SMITH. (Journal of Cancer Research, 1916, I, 231–258, 87 pl.)

The author reviews first the geographical and botanical distribution of crown gall. This disease is differentiated from hypertrophic enlargements in plants due to a few special parasitized cells as in the root nodules of legumes and slime mold infections in crucifers, as well as from granulomatous hyperplasia such as those of the olive tubercle where the bacteria are imbedded between the cells. Crown gall is a peculiar hyperplasia caused by *Bacterium tumefaciens* developing sparingly and only intracellularly, the parasitized cells being thereby caused to divide prematurely and repeatedly, which results in a great mass of non-capsulated small celled tumor tissue in which the bacteria themselves are invisible.

The tumor resembles cancer in many ways, notably in the exhibition of growth independently of function, vegetative activity being stimulated, functional activity depressed. The tumor can be stimulated by feeding or starved into quiescence. It can be grafted upon other plants of the same species. As in mammalian cancer, the nuclei of the tumor divide both mitotically and amitotically. Due to the rigid cell walls of plants, on the other hand, true metastasis seems not to occur.

The writer mentions especially the atypical arrangement of the tissues, their loss of polarity and the anaplasia, or undifferentiation of the cells. The production of tumors varying in structure according to the type of tissue experimentally invaded, among which were recognized embryonic organ inclusions analogous to the teratomata of mammals, is recorded for the first time.

The bacteriology of the parasite is discussed briefly, as are also some experiments with animals.—I. C. H.

PUBLIC HEALTH BACTERIOLOGY

Public Health Laboratories. R. G. PERKINS. (New York Med. Jour., 1916, **103**, 721–724.)

A discussion of the activities of public health laboratories. Among the suggestions made for furthering the development of these laboratories it is particularly recommended that there should be close association between the laboratory and a high grade university, in order that conditions should be made ideal for research work.—M. W. C.

The Microscopic Examination of Finger Nail Deposits. S. SCHNEIDER. (Jour. A. M. A., 1916. **66**, 1615–1617.)

The microscopic examination of finger nail deposits reveals the fact that the usual methods of cleansing the hands and nails does not result to any considerable degree in removing deposits.

Bacteriologic examination of deposits shows microorganisms to predominate in the following order: streptococci; staphylococci; *Bacillus coli;* bacilli, cocci, and spirillae derived from diverse sources; yeast

cells, spores and filaments of higher fungi; and larvae of vermes, amebas, diatoms, algae, etc.

Attention is called to the examination of such deposits from the medico-legal as well as from the hygienic standpoint.—G. H. S.

Controlling the Spread of Sputum. W. A. MANHEIMER. (Medical Record, 1916, **89**, 997–999.)

Sputum is one of the most dangerous of human discharges because of its wide dissemination and high content in pathogenic bacteria.

Experiments conducted to determine the viability of a culture of colon bacilli, when placed upon mailing envelopes as organisms in the sputum would be deposited in ordinary licking showed that of the bacteria sent through the mail, 5 per cent remained alive. Diphtheria bacilli placed upon envelopes and dried could not be cultivated in the few experiments performed for this purpose.

Valuable suggestions are given, which would aid in controlling the spread of sputum.—M. W. C.

MEDICAL BACTERIOLOGY

Case of Coccidioidal Granuloma. S. T. LIPSITZ, G. W. LAWSON, and E. M. FESSENDEN. (Jour. A. M. A., 1916, **66**, 1365–1367.)

Case report with detailed account of the blood picture and bacteriologic findings.—G. H. S.

The Contents of Ovarian Cysts. J. T. LEARY, H. J. HARTZ, and P. B. HAWK. (New York Med. Jour., 1916, **104**, 16–18.)

Bacteriological examination of the contents of six ovarian cysts resulted in negative findings in every case.—M. W. C.

Neisserian Proctorrhea. CHARLES C. MAPES. (The Urol. and Cut. Rev., 1916, **21**, 1.)

Infection of the rectal mucosa with the diplococcus of Neisser (*Gonococcus*) occurs more frequently than is generally believed.—C. P. B.

The Treatment of Human Rabies with Quinin and with Phenol. F. S. FIELDER. (Jour. A. M. A., 1916, **66**, 1300–1302.)

Several case reports of human rabies treated by injections of quinin or phenol. No specific action of the drugs was manifested.—G. H. S.

Trichinosis and the Cerebrospinal Fluid. W. LINTZ. (Jour. A. M. A., 1916, **66**, 1856.)

An accurate and rapid method of diagnosis in suspected trichinosis is to be found in an examination of the cerebrospinal fluid for *Trichina spiralis*.—G. H. S.

The Etiology of Typhus Fever in Mexico. (*Tabardillo.*) P. K. Olitsky, B. S. Denver, and C. E. Husk. (Jour. A. M. A., 1916, 66, 1792–1692.)
An organism was isolated from typhus fever patients in Mexico which possessed morphological and cultural characteristics identical with those of the *Bacillus typhi-exanthematici.*—G. H. S.

The Etiology of Iritis. E. E. Irons and E. V. L. Brown. (Jour. A. M. A., 1916, 66, 1840–1844.)
An etiologic study of 100 cases of iritis in which the causal relationships of syphilis, gonococcal infection, tuberculosis, dental, tonsillar, sinus, and genito-urinary infections are discussed.—G. H. S.

The Laboratory Examination of Material in a Case of Suspected Smallpox. J. N. Force. (Jour. A. M. A., 1916, 66, 1384.)
Pus obtained from a case of suspected smallpox injected intradermally into immune rabbits, and controlled by injections of vaccine virus, demonstrated the absence of small pox. The test required but forty-eight hours.—G. H. S.

Experimental Studies in the Production of Chronic Gastric Ulcer. A. O. Wilensky and S. H. Geist. (Jour. A. M. A., 1916, 66, 1382.)
Cultures of various strains of streptococci and yeasts derived from human gastric ulcers when injected into lesions artificially produced in the stomachs of cats failed to cause ulceration or retardation of healing.—G. H. S.

The Treatment of Genito-Urinary Tuberculosis with Rosenbach's Tuberculin. A. Hyman. (Jour. A. M. A., 1916, 66, 1379–1381.)
Rosenbach's tuberculin, a product of the symbiotic growth of the tubercle bacillus and *Trichophyton holosericumalbum*, was used therapeutically in thirteen cases of urogenital tuberculosis. Two cases showed improvement, the remainder were not influenced.—G. H. S.

Recurrent Generalized Herpes of Infectious Origin. F. Cohen. (Jour. A. M. A., 1916, 66, 1598–1599.)
Case report of generalized herpes simplex recurrent over a period of two years.
A streptococcus was isolated from the lesions and administered as a sensitized vaccine. Cure resulted.—G. H. S.

Streptothrix in Bronchopneumonia of Rats Similar to that in Rat-Bite Fever. R. Tunnicliff. (Jour. A. M. A., 1916, 66, 1606.)
A streptothrix, apparently *Streptothrix murisratti* was isolated from several rats affected with bronchopneumonia. Morphological and cultural characteristics are given.—G. H. S.

Control of Diphtheria. D. M. Lewis. (Jour. A. M. A., 1916, **66**, 1535–1536.)

Emphasis is placed upon the value of epidemiological work in the control of diphtheria. It is asserted that the examination of the naso-pharyngeal cavity of patients and contacts is more reliable than cultural work for the detection of carriers.—G. H. S.

Tuberculin in Surgical Tuberculosis. E. Bonime. (New York Med. Jour., 1916, **103**, 726–728.)

Tuberculin, if properly administered, is of great value in surgical tuberculosis. In cases where other infections occur in connection with the tuberculous processes, an autogenous vaccine should be used in addition to the tuberculin.—M. W. C.

Newer Laboratory Methods for the Early Diagnosis of Pulmonary Tuber-culosis. M. H. Kahn. (Jour. of Lab. and Clin. Med., 1916, **1**, 599–607.)

A description of various laboratory tests used in the diagnosis of tuberculosis. The only test of absolute diagnostic importance is the bacteriological examination of the sputum. All others are as yet of but relative value.—M. W. C.

A Case of Infection of Lymph Glands with Bacillus Paratyphosus B. C. S. Cole. (Jour. Infect. Diseases, 1916, **18**, 349–352.)

A condition of multiple lymph-adenitis, first diagnosed as Hodgkins' disease, and later found to be a paratyphoid infection without the manifestation of typhoid-like, gastro-enteric symptoms.—P. B. H.

Etiology and Laboratory Diagnosis of Smallpox and Chickenpox. J. N. Force. (Jour. of Lab. and Clin. Med., 1916, **1**, 243–251.)

A review of the methods which have been proposed for the diagnosis of smallpox and chickenpox.

For absolute diagnosis or differential diagnosis between smallpox and chickenpox, the intradermal inoculation of the suspected material into vaccinia immune rabbits appears to be the simplest and most reliable method.—M. W. C.

The Immune Response in Pulmonary Tuberculosis. E. Bonime. (New York Med. Jour., 1916, **103**, 930.)

In Germany the early use of tuberculin in tuberculosis has caused a marked diminution in the death rate from the disease. Physicians of this country should be urged to combine tuberculin treatment with the usual hygienic measures employed in the early stages of tuberculosis.

M. W. C.

Flagellate Protozoa as an Etiologic Factor of Dysenteric Diarrhea. B. W. Rhamy and F. A. Metts. (Jour. A. M. A., 1916, **66**, 1190–1191.)

The authors assert that *Trichomonas intestinalis* is a cause of acute or chronic diarrhea following the drinking of impure water.

This conclusion is supported by case histories and by the report of an epidemic consisting of 78 cases with 17 deaths.

Ipecac and emetin proved valuable in treatment.—G. H. S.

The Causation and Treatment of Pellagra. H. E. BOND. (Medical Record, 1916, **89**, 816–819.)

The theory is advanced that pellagra is caused by bacteria occurring in the intestinal tract. Toxins produced act primarily upon the sympathetic nervous system and secondarily upon the central nervous system. Treatment should consist of the internal use of gastrointestinal antiseptics and the external use of protective ointments.—M. W. C.

Notes on Grip Epidemic in Chicago. A. M. MOODY and J. A. CAPPS. (Jour. A. M. A., 1916, **66**, 1696.)

An analysis of 53 cases of grip shows that leucocytosis is usually absent. Bacteriologic examination of 31 cases gave the following results: *Streptococcus hemolyticus, Streptococcus viridans* and the pneumococcus were present 31 times; hemolytic staphylococci, 19 times; *Bacillus influenzae* and Friedländer's bacillus twice; *Streptococcus mucosus* and *Micrococcus catarrhalis* once.—G. H. S.

Antimeningococcic Serum in the Joint Manifestations of Gonorrhea. F. MALLETERRE. (New York Med. Jour., 1916, **103**, 1024–1026.)

Antimeningococcic serum is of value in generalized polyarticular forms of arthritis when several joints are involved and where there is moderate local inflammation without a fluid collection. The serum has little or no value in cases of gonorrheal monoarthritis with a large fluid collection.—M. W. C.

Pyelocystitis and Metastatic Abscesses Following Tonsillitis. H. B. MILLS and G. A. SOWELL. (New York Med. Jour., 1916, **103**, 725–726.)

Report of a case in which an attack of tonsillitis was followed by pyelocystitis and metastatic abscesses. *Staphylococcus albus* was the predominating organism isolated from the tonsils and was obtained in pure culture from the urine, blood and abscesses. The authors conclude that the tonsillitis was the etiological factor in the pyelocystitis, while the metastatic abscesses were complications of the latter.

M. W. C.

The Bacillus Epilepticus. C. A. L. REED. (Jour. A. M. A., 1916, **66**, 1607–1611.)

The author asserts that epilepsy is due to infection by *Bacillus epilepticus.* The organism is a spore bearer, whose primary focus of infection is the cecum but which under suitable conditions may invade the circulation.

The bacillus has been cultured from the blood and digestive tracts of epileptics and has proved pathologic for rabbits.—G. H. S.

The Diagnosis of Enteric Fever (Typhoid and Paratyphoid A and B) by Agglutination Tests. W. C. DAVISON. (Jour. A. M. A., 1916, **66**, 1297.)

Macroscopic agglutination tests made in accordance with the quantitative technic of Dreyer are reliable as diagnostic procedures.

In cases of suspected typhoid in persons who have received prophylactic inoculation a series of tests made at intervals of 5 to 10 days are necessary to establish the diagnosis.—G. H. S.

Notes on the Etiology of the Recent Epidemic of Pseudo-Influenza. W. W. WILLIAMS and W. BURDICK. (Medical Record, 1916, **89**, 876–877.)

A streptococcus was isolated from cases of pseudo-influenza, which, immediately after isolation, was virulent for mice and rabbits. Autopsy of rabbits, killed forty-eight hours after inoculation with the strain, revealed a tracheal and bronchial inflammation, suggestive of a selective action. After four or five generations of subculture, however, no such selective action was manifest. Upon artificial cultivation, the organism also reverted to the usual cultural characteristics.

M. W. C.

Bacteriology in Conjunction with Homeopathic Prescribing. W. W. IRVING. (Jour. of Oph., Otol. and Laryng., 1916, **22**, 490.)

The author understands the action of the homeopathic remedy to be much the same as that of vaccines, in stimulating the body tissues to greater resistance. "Or does the remedy stimulate the anti body function or does it act as a germicide?"

By a study based on the above theories he expects to produce a materia medica which will unify the organism with the remedy.—C. P. B.

Anopheles Punctipennis, a Host of Tertian Malaria. W. V. KING. (Am. Jour. Trop. Dis. and Prevent. Med., 1916, **8**, 426–432.)

In two comparative experiments *Anopheles punctipennis* was proven to be an efficient host for the *Plasmodium vivax.* These mosquitoes were bred from the larvae and pupae collected in the open. They were fed individually on a gametocyte carrier. A high percentage of infection was obtained. Controls of *A. quadrimaculatus* were also found to be infected. The infection in all instances was followed to the salivary glands.—F. M. J.

Simultaneous Injections of Streptococci and Dahlia in the Guinea-pig. W. H. HOFFMAN, W. B. MACLURE, and L. W. SAUER. (Jour. Infect. Diseases, 1916, **18**, 353–357.)

From a study of the effects of combined injections the authors found that an injurious reaction resulted, although no change was detected in the opsonic content of the serum or in the hemolytic reactions in which the serum was employed. They conclude that "intravenous injection is not warranted as a therapeutic measure."—P. B. H.

The Site and Rate of Destruction of Pneumococci Following Intraperitoneal Injection. F. BERRY and C. O. MELICK. (Journal of Immunology, 1916, **1**, 119.)

This report confirms the observations of Kyes concerning the mode of destruction of pneumococci injected into an unsusceptible organism (pigeon). The pneumococci as in Kyes' experiments were found to be taken up in great numbers by endothelial cells in the liver, and spleen. They appear in these cells within ten minutes after intravenous injection and within about two hours after intraperitoneal injection, and disappear completely in from twenty-four to thirty-six hours.—J. G. H.

A Final Report on the Cultivation of the Tubercle Bacillus from the Sputum by the Method of Petroff. R. A. KEILTY. (Jour. Ex. Med., 1916. **24**, 41–48.)

Cultures of the tubercle bacillus were obtained in 12 out of 18 positives cases of pulmonary tuberculosis and of 7 negative cases 1 showed growth. From 4 of the 12 positive cases pure cultures were obtained. In 12 cases sterile cotton swabs were rubbed over the tonsils, fauces, tongue and gums, the swabs treated with 3 per cent sodium hydroxide and the neutralized sediment inoculated on the Petroff medium; and in one case acid fast bacilli were obtained.—B. W.

Post-Operative Tetanus. KELLOGG SPEED. (Surgery, Gyn. and Obstet., 1916, **22**, 443.)

A very complete résumé of the literature. In addition six cases are reported all occurring after some abdominal operation. The author believes that some human beings are tetanus carriers and when operation is done, any injury to the wall of the intestines may result in tetanus through invasion from the intestinal contents.

The possibility of haematogenous infection must also be considered; likewise external contamination from fecal discharges may carry the infection.—C. P. B.

The Bacteriology and Experimental Production of Ovaritis. E. C. ROSENOW and C. H. DAVIS. (Jour. A. M. A., 1916, **66**, 1175–1180.)

The authors prove experimentally that streptococci derived from inflammatory conditions of human ovaries show an elective affinity for the ovaries of animals.

Cultures from ovaries removed at operation yielded *Streptococcus viridans* in a large number of cases. The gonococcus, the Welch bacillus, *Staphylococcus albus*, the colon bacillus and diphtheroid-like bacilli were also obtained.

Microscopic preparations of sections of the ovaries showed the presence of diplococci in several instances.

Cultures of the streptococci isolated were injected into rabbits and dogs and were recovered in pure culture from the ovaries of the animals.—G. H. S.

Elective Localization in the Bronchial Musculature of Streptococci from the Sputum of Cases of Bronchial Asthma. S. OFTEDAL. (Jour. A. M. A., 1916, **66**, 1693–1694.)

Cultures from the sputum of asthmatic patients injected intravenously into rabbits caused marked interference with the respiration. The respiration was reduced in rate and labored. Microscopic examination of the lungs showed marked distention of the alveoli with frequent rupture of the alveolar walls. The blood vessels were engorged. The bronchioles were contracted. Hemorrhage and mud cell infiltration were present. Streptococci were found in the musculature.

Tissue cultures of the lungs yielded pure cultures of streptococci. The author considers this an example of the elective localization of streptococci.—G. H. S.

An Epidemic of Appendicitis and Parotitis Probably Due to Streptococci Contained in Dairy Products. E. C. ROSENOW and S. I. DUNLAP. (Jour. Infect. Diseases, 1916, **18**, 383–390.)

The authors studied an outbreak occurring in a military academy in which fifteen cases of appendicitis and thirty-four cases of parotitis arose between February and the following May. From the appendix and tonsils of the patients streptococci were isolated that were pathogenic for rabbits, reproducing lesions in the appendix. Strains isolated from dairy products at the time of the epidemic produced similar lesions in rabbits. The authors conclude that the epidemic was caused by infected dairy products.—P. B. H.

The Tuberculocidal Action of Arsenic Compounds and Their Distribution in the Tuberculous Organism. A. ARKIN and H. J. COOPER. (Jour. Infectious Diseases, 1916, **18**, 335–348.)

The authors studied the effect of arsenic upon the tubercle bacillus and its distribution in the body. They report that (1) Sodium arsenite in dilutions from 0.1 to 0.0001 per cent and sodium cacodylate in dilutions of from 2.0 to 0.002 per cent had no germicidal action in twenty-four hours at 37°C., although mercury cacodylate showed germicidal power. (2) Atoxyl, arsacetin and neosalvarsan in dilutions of 1.0 to 0.001 per cent showed no germicidal power. (3) These compounds were found in the liver, lungs, kidneys, blood, spleen and in the tuberculous tissues (lymph glands and eye). No evidence of accumulation in the tissues was obtained.—P. B. H.

Syphilis in Epilepsy. W. T. SHANAHAN, J. F. MUNSON, and A. L. SHAW. (New York Med. Jour., 1916, **103**, 820–824.)

While syphilis may be considered one of the many agencies producing epilepsy, there is no special type of syphilitic epilepsy.

The percentage of syphilis in epileptic patients is approximately the same as the percentage in the general population.

Treatment of syphilis in epilepsy does not usually result in improvement, probably because permanent injury has been done to the tissues long before the beginning of treatment.—M. W. C.

Focal Sepsis. J. Daland. (New York Med. Jour., 1916, **103**, 1159–1160.)

Septic foci in the mouth, tonsils, sinuses, or prostate are frequently the cause of various systemic infections. The organism isolated from such foci is most frequently a streptococcus, usually *Streptococcus hemolyticus*. The severity of the systemic infection resulting from chronic focal sepsis varies with the virulence and number of the micro-organisms occurring in the focus, as well as with the resistance of the tissues to the spread of the infection.

The prompt diagnosis and removal of a septic focus is of the greatest importance.—M. W. C.

Meningitis. R. C. Rosenberger and D. J. Bentley. (New York Med. Jour., 1916, **103**, 1166.)

A report of seven cases, five of which were of the epidemic variety, one of pneumococcal, and one of tuberculous origin. The meningococcus was found in the spinal fluid of the five epidemic cases, and in one of the five a streptococcus was associated with the meningococcus. Cultures from a nasal discharge in two of the cases showed beside the usual bacterial flora, a gram negative diplococcus, which could not be differentiated from the meningococcus.

Antimeningococcus serum was administered to the five cases of the epidemic type. Three recovered, two did not.

Tubercle bacilli were demonstrable in the spinal fluid of the case with tuberculous meningitis two days before death. Pneumococci were present in the spinal fluid, and at autopsy, in the heart of the patient with pneumococcal meningitis.—M. W. C.

Tetanus: A Surgical Complication in the Present War. E. K. Tullidge. (New York Med. Jour., 1916, **103**, 1022–1024.)

The cases of tetanus treated during the present war, usually occurred as secondary infections, where the tetanus bacillus was found in wounded tissues in association with other organisms—most frequently with *Staphylococcus aureus* and *Bacillus aerogenes-capsulatus*.

The greatest mortality was in those cases of short incubation period, five days or less.

The most successful treatment was the administration of antitetanic serums in large doses, varying from 10,000 to 160,000 units.

Cases of long incubation period responded to smaller doses, but in cases displaying a short incubation period, large doses alone produce good results.

Chloral hydrate in doses of 5 to 10 grains was of great value in controlling convulsions. Cases where the wounds were treated locally with iodine did not develop the disease in its severest form.—M. W. C.

The Etiology of Common Colds. G. B. Foster. (Jour. A. M. A., 1916, **66**, 1180–1183.)

The conclusion that common colds are due to the action of a filterable virus is supported by the following observations:

The nasal secretions from individuals ill with colds were diluted with physiological salt solution and filtered through Berkefeld filters. The filtrates, when cultivated aerobically and anaerobically upon blood agar were sterile. Of ten men who were inoculated by placing some of the filtrate in the nostrils, nine developed colds.

The filtrates can be cultivated anaerobically in tissue—ascitic fluid. Stained preparations of the cultures were questionable but dark field examination showed the presence of active minute bodies possessing true motility.

Subcultures were prepared, filtered, and used to inoculate 11 men. After an incubation period of from eight to forty-eight hours all the men became ill with acute colds.

Filtrates from these experimentally produced colds could be cultivated.—G. H. S.·

The Production of Amyloid Disease and Chronic Nephritis in Rabbits by Repeated Intravenous Injections of Living Colon Bacilli. C. H. BAILEY. (Jour. Exp. Med., 1916, **23**, 773–790.)

The repeated intravenous injection of rabbits with living *Bacillus communior* over long periods has resulted in the formation of amyloid deposits in the spleen, liver, and kidneys. Suppurative lesions were not present in most cases and therefore not a factor in their production. The results have been constant in that amyloid was found in all rabbits, eight in number, which were injected over a period of eighty-eight days or more. Eight rabbits showed amyloid in the spleen, six of these in the kidneys also, and three in the liver.

The kidneys of these eight rabbits also showed as a result of the injections a subacute and chronic glomerulitis, parenchymatous degeneration, some interstitial infiltration with round cells, and a slight cellular proliferation of connective tissue, thus resembling the chronic parenchymatous nephritis of man which is so commonly associated with amyloid disease.—B. W.

Anthrax with Report of Cases. S. J. ULLMAN. (Surgery, Gyn., and Obstet., 1916, **22**, 450.)

Reports two fatal cases of anthrax occurring in negroes who had helped to skin the carcass of a cow. The animals on this pasture were vaccinated against anthrax but one cow had escaped while being driven up. Several months later it was found dead on the range. Showing no lesion it was ordered to be skinned. Two days after this one of the negroes was taken sick; he died on the eighth day of the disease, no physician having been called. The other negro was taken sick a week later. He was treated with anthrax vaccine and with serum from a horse inoculated against anthrax. but died one week after symptoms developed.

Two other negroes who helped do the skinning were taken sick two weeks afterward. Serum was given immediately; they recovered.

The author is not sure that these two had anthrax but does believe a standard serum is of considerable value.—C. P. B.

An Epidemic of Dysentery at Fort Shafter, Hawaii, with Three Cases of the Hiss-Russell or "Y" Bacillus Infection. G. M. VAN POOLE. (The Military Surgeon, 1916, **38**, 525–530.)

An Epidemic of Bacillary Dysentery due to the Hiss-Russell Bacillus. M. A. DELANEY. (Ibid., 531–533.)

Bacillary Dysentery, Recent Epidemic at Fort Shafter, H. T., from the Laboratory Aspect. F. H. FOUCAR. (Ibid., 534–538.)
This epidemic of 34 cases began on October 16, 1915, and the last case was received November 21, 1915. During this time 3 officers, 2 children, 27 enlisted men and 1 civilian employee were stricken. Two deaths resulted, thus giving a mortality of 6 per cent. The first of these papers discusses the epidemic from the sanitary standpoint, the second from the clinical standpoint and the third from the laboratory standpoint, thus giving a comprehensive study of a sharp outbreak of bacillary dysentery which was soon brought under control. Perhaps the most interesting point brought out is found in Captain Foucar's paper which states that a vaccine was prepared from the "Y" Bacillus isolated from these cases. The vaccine was made in accordance with the technique used in preparing the army typhoid vaccine, but was used in smaller doses. Three doses at ten day intervals were given, mostly to children, a total of 168 separate doses being given. Only one severe reaction occurred, and it is stated that the cases among the children ceased although adult cases continued for some time.—E. B. V.

Study of a Strain of B. Welchii Isolated in France Together with Some Notes on Gastric Ulcers. MARY W. STEWART and RANDOLPH WEST. (Journal of Immunology, 1916, **1**, 189.)
Stewart and West studied an organism isolated from gas gangrene which was a strict anaerobe, was capsulated, non-motile, and formed spores only in sugar-free broth containing coagulated egg white. It produced stormy fermentation of milk and in other respects conformed with the *B. Welchii* type of the butyric acid forming group of bacteria. Weinberg and Sacquépée have reported the formation of a soluble toxin by a gas gangrene organism which was motile and which sporulated on sugar media but Stewart and West were unable to detect a soluble toxin in the cultures of their bacillus. The killed suspensions of washed bacteria were harmless for guinea pigs. The filtrate of sugar broth cultures which was highly acid was toxic to guinea pigs but this toxicity could be completely removed by neutralization of the filtrate. The most noticeable lesions produced by these filtrates were acute gastric ulcers and such ulcers could be almost as regularly produced by the injection of acetic acid solutions of similar titre. The necrotic tissue at the site of an intramuscular inoculation with the bacilli was also found to be highly acid. Blood cultures from infected pigs were rarely positive.
The conclusion is drawn that the general toxic effects in gas bacillus

infections are due not to generalized infection or to a true toxin, but to the toxic effect of the acid produced at the site of the localized lesion.

                                                                    J. G. H.

Bacterial Cultures of Human Spleens Removed by Surgical Operation. ANDREW W. SELLARDS. (Journal of Immunology, 1916, 1, 321.)

Eight spleens from cases of pernicious anemia, one showing simple hypertrophy, and one from infantile primary splenomegaly, all removed at operation, were the subject of this study. Aerobic and anaerobic cultures were made on various media including milk and glucose ascitic agar. From four of the spleens micrococci, differing from the ordinary pyogenic types but not further identified, were recovered. From three of the spleens, small Gram positive pleomorphic bacilli were recovered in the anaerobic milk tubes. These organisms culturally and morphologically resembled a culture of the bacillus recovered from typhus by Plotz. Complement fixation reactions were carried out with extracts of these bacilli against the blood of rabbits immunized to them. Cross fixation was obtained between the antigens from the two spleen cultures tested, with the serum of the animal immunized to the Plotz bacillus, and an antigen prepared from the Plotz bacillus gave fixation with the serum prepared by injection of the spleen cultures. Out of eight human sera tested, one from a case of pernicious anemia, and one from a case of cholangitis gave complete fixation with spleen bacillus antigen and with Plotz bacillus antigen. Other sera were negative. In fermentation tests, two of the spleen cultures differed from the bacillus of Plotz in fermenting mannite and in failing to ferment inulin, and one differed only in the absence of inulin fermentation. Sellards concludes that these organisms represent parasitic but non-pathogenic organisms found in the human body.—J. G. H.

Bacteriological and Experimental Studies on Gastric Ulcer. H. L. CELLER and W. THALHIMER. (Jour. Exp. Med., 1916, 23, 791–812.)

Eight chronic gastric ulcers and one ulcer occurring at the ostium of a gastrojejunostomy were examined bacteriologically and histologically. From seven of these anhemolytic streptococci were isolated; streptococci were seen in cultures of the eighth but could not be isolated and from the ninth no streptococci were recovered. Yeasts were recovered from four. Other organisms including staphylococci, *Micrococcus tetragenus* and *B. subtilis* were isolated. The streptococci isolated were injected into rabbits and cats intravenously. Of thirty rabbits injected in the ear vein, four developed gastric lesions. Of eight rabbits injected in a branch of the gastric artery six developed gastric lesions. In two cats a branch of the gastric artery was injected with streptococci. Both animals developed defects in the gastric mucosa, which soon began to heal and were observed to have healed completely in thirty-three days. In addition fourteen of the thirty rabbits developed cardiac lesions, while two of the eight in which a branch of the gastric artery was injected developed minute hemorrhages in the endo-

cardium.   The authors are unable to decide definitely whether or not
the gastric lesions produced by the injection of the rabbits with strep-
tococci are to be considered ulcers.   From their experiments they con-
clude "It must be assumed that some cause is operative in certain
cases preventing the healing of defects in the gastric mucosa and is
inoperative in others.   Even though anhemolytic streptococci are
present in practically all gastric ulcers, we cannot convince ourselves
that these organisms have been proven as yet to be the factor which
either initiates the ulceration or prevents healing.   Nevertheless, the
constant presence of streptococci in this type of lesion is a suggestive
fact and further experiments to determine their significance are being
undertaken."—B. W.

STUDIES IN THE NOMENCLATURE AND CLASSIFICATION OF BACTERIA

THE PROBLEM OF BACTERIAL NOMENCLATURE[1]

R. E. BUCHANAN

From the Bacteriological Laboratories, Iowa State College, Ames, Iowa

Erwin F. Smith in the chapter on "Nomenclature and Classification" in the first volume of his work on Bacteria in Relation to Plant Diseases (1905), accepts as valid 33 different names of bacterial genera. In addition he presents a list of 156 generic names which he regards as definitely invalid or inappropriate and to be rejected. In the preparation of material for a course in systematic bacteriology given at Iowa State College for several years, I have had occasion to use this list, and have added to it. It appears that about 300 generic and pseudogeneric names have been used by bacteriologists. In addition, about 100 names have been used for orders, classes, families, subfamilies, tribes, and subtribes. The problem as to which of these names are to be regarded as valid, and which invalid, has been increasingly emphasized as one of considerable importance, and intimately associated with the development of a satisfactory classification of the bacteria.

To state that the classification of bacteria is in a chaotic condition is to express a truism. That this is due to the inherent difficulties in determining bacterial relationships, and to the utter disregard of all rules of nomenclature is likewise generally accepted. Is there any need of action?

Of recent years some bacteriologists have apparently taken delight in ignoring the well established customs of biological nomenclature, and in creating special rules to fit occasions. In

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this they seem in many cases to have been aided and abetted by our technical and scientific periodicals and their editors, particularly those in the medical or medico-scientific field. Occasionally an effort is apparently made to depart as far as practicable from good usage. For example, it is customary in both botanical and zoölogical periodicals and generally in the literature of these sciences to treat the name of a genus as a proper name, and to capitalize it at least when used with a specific name. The rule reads, "Genera receive names, substantives in the singular number and written with a capital letter." A perusal of technical and medical journals shows the rule to be commonly ignored. This fact in itself is probably not of great importance, but is a symptom of a deep seated trouble. Everywhere we find disregard of law and precedent, and everywhere the loose thinking and writing which are the consequence.

The whole subject of formal nomenclature, notwithstanding current lack of interest, is of great importance from the standpoint of bacteriologists, sanitarians, hygienists, physicians and pathologists. Our present system, or lack of system, leads to inaccuracies, misconceptions, and misstatements. We can not long continue to violate the principle that for every kind of living thing there shall be a single valid name, without causing confusion.

The subject of nomenclature is in part distinct from that of classification. The latter deals with methods and criteria, of use in the differentiation of groups from each other, the former has for its function the determination of the kind of a name that shall be applied to a particular group, and the validity and suitability of names that have already been applied.

The efforts of the Society of American Bacteriologists have been directed in the main in the past to the problems of differentiation of kinds and groups of organisms, as witnessed by its descriptive chart. It is time that it should bestir itself concerning the labels it is to place on the groups which it is learning to separate. It is probably safe to state that practically every other branch of biological science has left us in the rear in this matter.

Perhaps our fundamental difficulty is to know whether we, as bacteriologists, are zoölogists or botanists. Both of these groups of scientists have formulated and revised from time to time in international congresses, elaborate codes of nomenclature, not perfect perhaps, but helpful and necessary. A study of the writings of protozoölogists, helminthologists, and those dealing with microscopic forms of life definitely on the zoölogical side of the fence will show that they have applied their code with a considerable degree of satisfaction; information in regard to their subjects can, in consequence, be quite satisfactorily systematized. The algologists, mycologists, and most of the botanists, have likewise worked out their schemes of classification in accordance with definite rules. The bacteriologists on the other hand have thus far failed to agree on any scheme of classification, and many are rebellious against any of the restraints imposed by formal nomenclature. Frequently nomenclature and classification have been confused in our minds. We have been unable in many cases to define what is meant by the term bacterial species, we hesitate to give a name to that which we can not accurately define. But it is equally true that species have not been accurately delimited in the older biological sciences, and this fact has not interfered with at least tentative arrangements of genera and higher groups, nor with the application of correct names.

A careful search of our literature fails to show that any group of bacteriologists has thus far formally agreed upon any code or system of nomenclature. Inasmuch as the bacteria are to be regarded as plants, the natural suggestion is that we should follow the botanists' code. But within recent years bacteriologists have shown a tendency to term themselves microbiologists in order to make it evident that a portion of the zoölogical field is to be covered as well. Furthermore there is no perfect accord as yet as to where certain microörganisms belong; forms such as spirochetes, which certainly are within the province of bacteriology, may be regarded as either animals or plants.

A careful study of the botanical and zoölogical codes will show them to be very similar in most essential characteristics.

The botanical code is less rigid, and probably has something more of ambiguity in some of its statements than does the zoölogical. Logically it would seem that we should follow the botanical code with the bacteria and the zoölogical code with the protozoa.

The questions at once arise:

Are these codes really applicable to the nomenclature of microörganisms? Have they not been planned with higher groups of plants and animals in mind?

A careful study of the provisions of either code will show that there is no inherent difficulty in application to the lower forms of life. In the botanical code there are perhaps, one or two rules which the bacteriologists might be reluctant to accept, particularly the rule that all new species of plants (in our case, bacteria) to be recognized as valid, must be published with a Latin diagnosis. Furthermore in both botanical and zoölogical codes there is a rule that no two genera (or other groups) can exist with the same name, and that duplicate genera in plant and animal kingdoms should be avoided as far as possible, but that such duplicates are not invalid. In other words, two plants can not have the same name, nor can two animals, but a plant and an animal may be named alike. It would appear that for the sake of the microbiologist there might be appended to each code the rule that in those groups whose position in plant or animal kingdom is in doubt there should exist no duplicate names.

At the last botanical congress held in 1910, certain points relative to bacterial nomenclature were definitely referred to a congress which was to have been held in the summer of 1915, but which was deferred because of the war. At this congress two points of interest to bacteriologists were to have been taken up: 1, the determination of the time or point of departure in nomenclature of the Schizomycetes, and 2, the adoption of a list of *genera conservanda.*

In preparation for this congress Vuillemin (1913), published a paper in which he discussed bacterial classification and nomenclature. He concludes that the best thing to do is to determine

which generic names are valid, prepare such a list, publish the names as *genera conservanda*, and date all bacterial classification from 1915 when the list should have been adopted by the congress. He comes to the conclusion that all the true bacteria should be included in the following genera: *Planococcus, Streptococcus, Klebsiella, Merista, Planomerista, Neisseria, Sarcina, Planosarcina, Metabacterium, Clostridium, Serratia, Bacterium* and *Spirillum*.

A study of Vuillemin's paper, despite his criticism of the bacteriologists as taxonomists, shows that he himself does not formulate tenable bases for differentiation of genera, and his reasons for choosing certain generic names and abandoning others will scarcely withstand critical analysis.

But after all, is a scientific classification of the bacteria important and desirable? Allow me to quote from a paper published about two decades ago by H. Marshall Ward. He says:

The only really valid objection to a purely scientific classification is the old objection of the purely utilitarian "practical" man; and even there the objection is relative. This leads me to bring out the point that the bacteriologists in the widest sense of the word, are really looking at the question of classification from at least two very different points of view: On the one hand, we have the botanists, who direct their attention to the organism, the Schizomycete itself, as a biological phenomenon to be examined and reported upon as thoroughly as possible, for them no classification is complete which does not record, or (which amounts to the same thing) imply in its records, all of the life phenomena of the organism including its pedigree.

On the other hand, we have the pathologists, hygienists, brewers, chemists, etc., who regard the organism simply as an object to be named for convenience in reference, because it brings about certain changes in the tissues, waters, and other media which they are more specially concerned with. They do not care, and naturally so, what vagaries the organism exhibits, so long as they can recognize it when they meet with it. As a matter of experience, however, it is just these vagaries that bring about the sources of error which beset them on all hands, and hence they are equally interested with the botanist in having them cleared up and explained. When we come to the conclusion that, whatever may be believed to the contrary, the real interests of "bacteriologists" of all kinds are identical.

Are we yet ready to make a real, systematic, effort to classify and name the bacteria? As a society we are committed to the idea that we *are* ready, as shown by our possessing a committee on classification. It would seem that just now the time is propitious for the careful formulation of general rules of bacteriological nomenclature and of a scheme of bacterial classification.

What can we do? Much, it would appear, through the aid of the committee on classification or a new committee on nomenclature or both.

We can ask that the committee make a careful study of the botanical, and perhaps, too of the zoölogical codes of nomenclature. They could then report what changes, if any are necessary to make either serve as a working scheme for bacteriology.

They could make recommendations as to the date of departure for bacterial classification.

They could report on the historical validity of the names that are used for bacterial groups, particularly genera, and determine their type species, and adequate diagnoses.

They could prepare a list of recognized generic names, such as that maintained in the check list of birds by the ornithologists of this country.

They could seek the active coöperation of committees of other societies interested in like problems in this country, and as rapidly as practicable, work with similar organizations in other countries.

They could prepare a set of resolutions for the next international botanical congress that would doubtless contribute greatly to the solution of the problem of nomenclature and classification among the bacteria.

And lastly, they might with profit give some careful thought to the preparation of a classification of the chemical changes brought about by microörganisms. It seems that we are fated to hear and read discussions of azofication, ferrification, etc. Could not a system etymologically sound be worked out for such terms in the interest of uniformity?

REFERENCE

VUILLEMIN. 1913. Genera Schizomycetum. Annales Mycologici. **11**, 512-527.

THE OXYGEN REQUIREMENTS OF BIOLOGICAL SOIL PROCESSES[1]

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The aim of this work was to find out whether the fundamental processes carried on by soil bacteria proceed better under aerobic or anaerobic conditions.

The work was carried out with three soils, first a greenhouse loam soil, rich in organic matter, second a field soil from the experiment station plats,—a Hagerstown silt loam, and third, a clay soil—a Hagerstown clay taken from the side of a hill sloping down to a brook. These were selected to represent three different types of soil and to secure different flora and different conditions of microbic development.

The biological processes in soil are influenced according to Lipman (1911) by moisture, temperature, aeration, reaction, and food supply. It seems to the writer that the relation of oxygen to the fundamental soil processes has not been thoroughly investigated; and that it has not been fully established—taking soil or synthetic solutions as media—whether nitrogen fixation, nitrification, ammonification and denitrification will go on under aerobic or anaerobic conditions only, and whether these processes will take place better in the presence or in the absence of air.

Preliminary experiments showed that ammonification, denitrification, and nitrogen fixation took place readily with or without air. Nitrification on the other hand would not take place under anaerobic conditions, either in soils or in solution in the preliminary or subsequent experiments. The results of the work on nitrification are therefore not included in this paper.

[1] Paper No. 45 from the Laboratories of Plant Pathology and Bacteriology, Va. Agr. Exp. Sta.

AMMONIFICATION

Kelley (1915) found that anaerobic conditions greatly retard the formation of ammonia from all materials except casein and under anaerobic conditions the formation of ammonia has usually been found to be considerably less than under aerobic conditions. Aeration (Kelley, McGeorge and Thompson, 1915) stimulates ammonification but is not essential to the process as it is to nitrification.

Lohnis and Green (1913) found that aeration is of great importance in the ammonification of organic matter. "The most significant cause of variation appears to be that of aeration. Ammonification as a whole proceeds much more rapidly under aerobic than under anaerobic conditions, but it is believed that aerobic conditions favor more specifically those latter stages in the breakdown, which result in the formation of ammonia itself." Later (Lohnis and Green, 1914) they report that while aeration is not of preponderating importance ammonification of such substances as flesh meal, bone meal and blood meal proceeds better under aerobic than anaerobic conditions.

In my experiments the process of ammonification was tested both in soils and in solution. One hundred grams of soil of the different varieties was inoculated with 1 gram of the ammonifiable substance. Blood meal sterilized with CS_2 and sterile casein (Brown, 1913) solution were used. For solution work, Dunham's solution (1 per cent peptone plus 0.5 per cent salt) and urea bouillon (nutrient bouillon and 1 per cent urea) were used. In each case an easily and a less readily ammonified substance were used, casein and urea being easily ammonified.

Anaerobic conditions were obtained with the soils by placing them under a bell jar in a somewhat larger dish with pyrogallic acid and caustic soda solution. The pyrogallic acid was placed in the bottom of the dish, the soil in tumblers, and the bell jar put on, the caustic soda solution added, and then some paraffin oil. The bell jar was lifted slightly to let the caustic soda come in contact with the pyrogallic acid. The oil prevented the mixture from absorbing oxygen from the atmosphere. In solution

anaerobic conditions were obtained by adding one inch of sterile paraffin oil. The materials were incubated a week at 30° C. and the ammonia determined by distillation with MgO.

Experiment I. Ammonification of blood meal and casein under aerobic and anaerobic conditions in soils. Ammonia in milligrams per 100 grams of soil.

Casein

SOIL	AEROBIC	ANAEROBIC
Greenhouse..................................	90.10	97.26
Loam...	78.05	77.46
Clay..	71.25	43.18

Blood meal

SOIL	AEROBIC	ANAEROBIC
Greenhouse..................................	21.42	20.70
Loam...	21.08	11.90
Clay..	10.88	7.60

Experiment II. Ammonification in sterile soil by mass cultures under aerobic and anaerobic conditions. Ammonia in milligrams per 100 grams of soil.

Casein

SOIL	AEROBIC	ANAEROBIC
Greenhouse..................................	96.39	95.61
Loam...	82.96	81.26
Clay..	91.29	81.94

Blood meal

SOIL	AEROBIC	ANAEROBIC
Greenhouse..	98.94	105.23
Loam...	70.72	87.72
Clay..	8.89	14.62

In experiment II the mass cultures were obtained by inoculating Lipman's synthetic media (Lipman and Brown 1911) with soils of the different types and growing one week at 30° C. The soil was sterilized in the autoclave. Twenty cubic centimeters of this mass culture were added to each 100 grams of soil.

It is at once apparent that ammonification in soil proceeds just as readily under anaerobic conditions as it does under

aerobic conditions. The process in general proceeds better in richer soils. It is best with the greenhouse type and poorest with the clay. Casein is more easily broken down than blood meal. Blood meal is ammonified to a greater extent under aerobic and anaerobic conditions in sterile inoculated soil than in fresh soil. The soils kept under anaerobic conditions gave a strong disagreeable odor, showing that other processes were going on. This was not noticeable with the soils kept under aerobic conditions.

Experiment III. Ammonification in solution. Ammonia in milligrams per 100 cc. of solution.

Urea bouillon

SOIL	AEROBIC	ANAEROBIC
Greenhouse....................................	554.71	679.66
Loam..	448.75	687.65
Clay..	553.69	684.22

Dunham's solution

Greenhouse....................................	136.85	141.78
Loam..	134.30	125.12
Clay..	147.90	148.24

One hundred cubic centimeters of the solutions in 250 cc. Erlenmeyer flasks were inoculated, respectively with 2 grams of soil of each type. One inch of sterile paraffin oil was added to half of them, the other half being kept under aerobic conditions.

Ammonification in solution goes on under anaerobic conditions as well as under aerobic conditions. The urea is more easily ammonified than the peptone. The ammonification of urea proceeds better under anaerobic conditions than under aerobic conditions. The ammonification of peptone proceeds just as readily in the presence as in the absence of air. Larger amounts of ammonia were formed in solution than in soil.

It was thought that an excess of air might inhibit or increase the amount of ammonia formed. A preliminary experiment was carried out by bubbling washed air through 100 cc. of Dunham's solution inoculated with 2 grams of garden soil (greenhouse).

Air was bubbled through sterile water, then through the Dunham solution, and then through 50 cc. of $\frac{N}{10}$ H$_2$SO$_4$, colored with methyl red, by means of a water air pump. Any ammonia that was drawn across in the process was caught in the acid. As the acid lost color, more acid was added.

Milligrams of ammonia formed per 100 cc. Dunham's solution

No. I. Excess air.. 120.53
No. II. Air... 155.72
No. III. Without air (oil). 163.20

From these data it would appear that ammonification proceeded best without air, next with air and least with an excess of air.

Experiments were carried out with urea bouillon (no peptone) and Dunham solution with the bacteria of the three types of soil, under anaerobic, aerobic and excess aerobic conditions.

Experiment IV. Ammonification in solution. Milligrams ammonia per 100 cc. of solution.

Urea bouillon

SOIL	EXCESS AIR	AIR	WITHOUT AIR
Greenhouse....................	357.85	313.48	570.80
Loam..........................	302.94	306.51	563.04
Clay..........................	291.29	271.53	550.80

Dunham solution

Greenhouse....................	130.17	149.36	161.23
Loam..........................	145.71	148.89	154.17
Clay..........................	55.11	93.31	137.17

It is again noticeable that urea is more readily ammonified than peptone. Ammonification proceeds best under anaerobic conditions. The process seems to proceed equally well with air or with an excess of air. With urea bouillon the production of ammonia is slightly higher with an excess of air than under ordinary air conditions. With Dunham's solution the production of ammonia is slightly higher without an excess of air.

Some further experiments with pure cultures were carried out. A pure culture was isolated from an ammonified urea solution and grown on urea agar under anaerobic conditions. A very simple method was devised for anaerobic plate work. It consisted in adding sterile paraffin oil to agar that had been cooled, inoculated and poured. Oil was added to the level of the rim of the plate. This avoided the use of the anaerobic jar and proved very effective. No spreading colonies were observed, and colonies were as well isolated as on an aerobic plate. The plates may be removed from the incubator and examined for growth at any time. This is a decided advantage over the anaerobic jar method. The oil may be poured off the plate and the colonies exposed for further study.

The organism isolated by this method was a diplo-bacillus. It would not grow on nutrient agar under aerobic or anaerobic conditions. It grew very well on urea agar under aerobic and anaerobic conditions, although it had been isolated purely by anaerobic technique.

Cultures of this organism were inoculated into 100 cc., respectively of urea bouillon (no peptone), urea solution (glucose, 10 per cent, K_2HPO_4 5 per cent, $MgSO_4$ 0.05 per cent, urea 1 per cent), and Dunham solution. These inoculated flasks were placed under aerobic and anaerobic conditions.

Experiment V. Ammonification in solution by pure culture. Ammonia in milligrams per 100 cc. of solution.

MEDIA	AEROBIC	ANAEROBIC
Urea bouillon....................................	308.69	530.53
Urea solution....................................	130.63	79.31
Dunham solution.................................	—	—

In this experiment urea bouillon seemed to be the best medium. Ammonification with this material proceeded better under anaerobic conditions. The reaction did not take place with Dunham's solution. It did not proceed as well with the urea solution as with urea bouillon, although the process went on under aerobic and anaerobic conditions.

Some further experiments were carried out with pure cultures of *B. mycoides* and *B. subtilis* freshly isolated from the soil.

Experiment VI. Ammonification in solution by B. mycoides and B. subtilis. Ammonia in milligrams per 100 grams of solution.

Urea bouillon

ORGANISM	AEROBIC	ANAEROBIC
B. mycoides....................................	42.46	62.19
B. subtilis	41.28	62.67

Dunham solution

B. mycoides....................................	35.71	14.37
B. subtilis	9.95	6.07

These two facultative anaerobes not only live under anaerobic conditions, but carry out their activities as well. Urea bouillon is more easily ammonified than the Dunham solution by these organisms. More ammonia is produced under anaerobic conditions with urea and less with peptone.

In general, from a perusal of the preceding experiments, it appears that ammonification of the substances tested under laboratory conditions, proceeds readily under aerobic or anaerobic conditions in mass cultures using soil as a medium or in media inoculated with soil or when pure cultures isolated from the soil are used.

The ammonification of blood meal and casein proceeds as well under anaerobic as under aerobic conditions in the soil. The same is true of ammonification in solutions of urea and peptone. More urea is, however, broken down under anaerobic conditions. Excess air bubbled through inoculated liquid media does not inhibit the production of ammonia, although less ammonia was produced with Dunham's solution under these conditions than under ordinary air conditions. Pure cultures of *B. mycoides* and *B. subtilis* readily form ammonia under anaerobic conditions. More ammonia is however produced from urea under anaerobic conditions by these organisms.

NITROGEN FIXATION

The ability of microörganisms to fix atmospheric nitrogen was first definitely demonstrated to be due to an anaerobic bacillus, *B. Pasteurianus*, in 1893 by Winogradski. It remained for Beyerinck in 1901 to demonstrate an aerobic organism that also assimilated free nitrogen. Non-symbiotic fixation of nitrogen in the soil is due to both types of organisms but at times may be due only to one type. Lipman and Burgess (1915) found two-thirds of the soils examined by them free from *Azotobacter*. Yet these soils were capable of fixing nitrogen when inoculated into solutions. They ascribed the nitrogen fixation to clostridium forms. Haselhoff and Bredemann (1906) investigated anaerobic nitrogen-collecting bacteria and found results approximating those of Winogradski. The amount of nitrogen fixed varies with the amount of carbonaceous matter present, the more carbon the higher the assimilation. Working with pure and mixed cultures, they found from 0.42 to 2.74 mgm. of nitrogen fixed per gram of mannite. Lipman (1908), working with pure cultures of *Azotobacter*, found from 0.39 to 10.45 mgm. of nitrogen per gram of mannite formed in four weeks in mannite solution. In this work mass cultures were used, either by inoculating solution with soil or by adding the source of carbon to the soil.

In my work nitrogen fixation was carried out in soils and in solution. For the solution work, 100 cc. of a nitrogen poor medium was inoculated with from 2 to 5 grams of soil. The following solution (N. J. 1908) was used:

H_2O .... 1000.0 grams
K_2HPO_4 .... 0.2 gram
$MgSO_4$ .... 0.2 gram
$NaCl$ .... 0.5 gram
Mannite .... 20.0 grams
$FeCl_3$ .... 1 drop of 10 per cent solution

The solution was neutralized with KOH using phenolphthalein as an indicator. Anaerobic conditions were obtained by adding about an inch of sterile paraffin oil or by placing the material in the anaerobic apparatus described under the ammonification experiments.

For nitrogen fixation in soils, the three types of soil used for ammonification were again studied. One gram of mannite was added to 100 grams of soil in a beaker. Anaerobic conditions were again obtained by the absorption of oxygen with pyrogallic acid and caustic soda solution.

The materials were incubated at about 30° C. for twenty-one days and then the total nitrogen was determined by the modified Gunning method, (Hibbard, 1910). Blanks were run at the beginning and the difference between these blanks and the total nitrogen at the end of twenty-one days gives the amount of nitrogen fixed.

Experiment VII. Nitrogen fixation in solution. Nitrogen in milligrams per 100 cc. of solution.

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN
Aerobic			
Greenhouse.....................	12.00	9.38	2.62
Loam.........................	7.60	3.75	3.85
Clay..........................	5.60	1.74	3.86
Anaerobic			
Greenhouse.....................	12.60	9.38	3.22
Loam.........................	6.30	3.75	2.55
Clay..........................	5.10	1.74	3.36

In this experiment 100 cc. of the solution contained in 250 cc. Erlenmeyer flasks was inoculated with 5 grams of the soils of the different types. About one inch of sterile paraffin oil was added in order to insure anaerobic conditions.

All of the soils used are capable of fixing nitrogen under both aerobic and anaerobic conditions. The greenhouse type of soil is richer than the loam and the loam richer than the clay in total nitrogen both at the beginning and at the end of the experiment. The actual increase in nitrogen does not vary much with any of the three soils. Lipman and Burgess (1915) also noticed this and remarked in a conclusion that as a rule a high nitrogen content in the soil seems to mitigate against a vigorous nitrogen fixation. The nitrogen fixation seems to proceed as readily with or without the presence of air in this experiment.

In the following two experiments nitrogen fixation was carried out in solution under anaerobic conditions by placing narrow bottles containing the mannite solution, inoculated with 5 grams of soil, in the anaerobic apparatus and absorbing the oxygen with pyrogallic acid and caustic soda solution. In one case thin bottles containing a solution in which denitrification was going on, were added.

Experiment VIII. Anaerobic nitrogen fixation in solution. Nitrogen in milligrams per 100 cc. of solution.

A

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN
Greenhouse.....................	11.34	9.38	1.96
Loam..........................	7.00	3.75	3.25
Clay...........................	8.68	1.74	6.94

B

Greenhouse.....................	9.80	4.18	5.62
Loam..........................	12.88	1.50	11.38
Clay...........................	5.18	0.70	4.58

In Series B denitrification was going on in the same apparatus in three other samples. The nitrogen fixing solution was inoculated with 2 grams of the soils. There was more nitrogen fixed in B, probably due to the fact that nitrogen was being continually given off in the denitrification experiments. This might indicate that the more nitrogen present the more nitrogen is fixed. It may be that as denitrification takes place in the soil some part of this nitrogen may be again fixed immediately. Except in the last experiment anaerobic conditions do not seem to favor the production of more nitrogen than is produced under aerobic conditions in solutions. Nitrogen fixation in solution proceeds as well with or without the presence of air.

Further experiments were carried out in soils by adding 1 gram of mannite per 100 grams of soil. Anaerobic conditions were obtained with pyrogallic acid and caustic soda solution. The total nitrogen was determined in 10 gram samples at the beginning and end of the experiment.

Experiment IX. Nitrogen fixation in soils. Nitrogen in milligrams per 10 grams of soil.

Aerobic

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN
Greenhouse....................	27.16	26.32	0.84
Loam..........................	12.46	9.38	3.08
Clay..........................	5.18	4.34	0.84

Anaerobic

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN
Greenhouse....................	34.82	26.32	8.50
Loam..........................	14.66	9.38	5.29
Clay..........................	9.03	4.34	4.69

In these experiments greater amounts of nitrogen are fixed under anaerobic conditions than under aerobic conditions. There is a gradation shown under anaerobic conditions, most nitrogen being fixed by the greenhouse soil, less by the loam and least by the clay.

In the following experiment soil was sterilized in the autoclave and mass cultures grown under aerobic and anaerobic conditions in mannite solution were added.

Experiment X. Nitrogen fixation in sterile soil. Nitrogen in milligrams per 10 grams of soil.

Aerobic

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	LOSS OR GAIN
Greenhouse....................	24.67	26.32	−1.65
Loam..........................	12.50	9.32	3.10
Clay..........................	5.60	4.34	1.26

Anaerobic

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	LOSS OR GAIN
Greenhouse....................	27.86	26.32	1.54
Loam..........................	11.48	9.38	2.10
Clay..........................	5.04	4.34	.70

The number of bacteria per gram of soil, capable of growing on nitrogen poor media, was estimated on three different media under aerobic and anaerobic conditions. The following media were used:

Mannite agar		*Ashby agar*		*Winogradski agav*	
K_2HPO_4.........	0.2	K_2HPO_4.......	0.2	K_2HPO_4....	1.00 gram
$MgSO_4$..........	0.2	$MgSO_4$.........	0.2	$MgSO_4$.....	3.00 grams
NaCl............	0.5	NaCl..........	0.2	NaCl......	0.01 gram
H_2O............	1000.0	$CaSO_4$........	0.2	$MnSO_4$.....	0.01 gram
Mannite........	20.0	$CaCO_3$........	5.0	$CaCO_3$.....	10.00 grams
Agar............	15.0	H_2O...........	1000.0	$FeCl_3$.2 drops 10 per cent	
$FeCl_3$, 1 drop 10 per cent		Agar..........	15.0	H_2O........	1000 grams
Neutralized with KOH.				Agar......	15 grams

The soil was plated and the plates were incubated at 30° C. under aerobic and anaerobic (pyrogallic acid and caustic soda) conditions for seven days and then counted.

Experiment XI. Number of bacteria per gram on nitrogen-poor media.

Aerobes

SOIL	MANNITE AGAR	WINOGRADSKI AGAR	ASHBY AGAR
Greenhouse....................	26,000,000	29,000,000	4,000,000
Loam.........................	1,150,000	1,000,000	1,190,000
Clay.........................	40,000	21,000	32,000

Anaerobes

Greenhouse....................	1,050,000	1,020,000	310,000
Loam.......................	240,000	240,000	190,000
Clay.........................	11,800	9,600	3,500

Greater numbers of bacteria develop under aerobic than anaerobic conditions. There are more aerobic than anaerobic bacteria capable of growing on nitrogen-poor media. The number of bacteria varies with the type of soil, the greatest number being present in the greenhouse type and fewest in the clay.

Two cultures of *Azotobacter* were picked from the aerobic plates and two cultures were picked from the anaerobic plates. These cultures were inoculated into 100 cc. of nitrogen poor media,—Mannite solution, Winogradski solution and Ashby solution (same composition as the agars without the agar). The two cultures taken from the anaerobic plates were kept, with and without, oil. All inoculated material was incubated twenty-one days at 30°C.

Experiment XII. Nitrogen fixation in solution by pure cultures. Nitrogen in milligrams per 100 cc. of solution.

Aerobes

MEDIA	CULTURE NUMBER	NITROGEN FIXED
Mannite.......................................	1	2.02
	2	1.86
Ashby...	1	3.23
	2	1.86
Winogradski..................................	1	3.23
	2	3.39

Anaerobes (oil)

MEDIA	CULTURE NUMBER	NITROGEN FIXED
Mannite.......................................	3	
	4	1.75
Ashby...	3	1.90
	4	1.75
Winogradski..................................	3	2.02
	4	2.21

Experiment XIII. Nitrogen fixation in solution by pure cultures. Nitrogen in milligrams per 100 cc. of solution.

Anaerobes

MEDIA	CULTURE NUMBER	NITROGEN FIXED UNDER OIL	NITROGEN FIXED WITHOUT OIL
Mannite.......................	3	—	2.63
	4	1.75	2.63
Ashby........................	3	1.90	2.32
	4	1.75	2.39
Winogradski..................	3	2.02	7.21
	4	2.21	3.54

More nitrogen is fixed by the anaerobic organisms. More nitrogen is fixed by the anaerobic organisms when inoculated into media with no oil added to insure anaerobic conditions than when oil is added. In the last case more nitrogen is fixed by the anaerobes without oil than is fixed by the aerobes. The Winogradski medium seems to be the best for nitrogen fixation by pure cultures. More nitrogen is fixed in Winogradski media than in the other two materials used.

In general, fixation proceeds better in soils than in solution, more nitrogen being fixed in soils. The nitrogen fixed per gram of mannite is higher with the soils than in solution. The greater amount of nitrogen fixed in soils may be due of course to other forms of energy in the soil in the shape of decomposed plant tissue. Nitrogen is fixed readily under aerobic or anaerobic conditions in solutions. In soils, nitrogen fixation proceeds better under anaerobic conditions.

DENITRIFICATION

Broadly speaking, denitrification is the breaking down of nitrates to nitrites and ammonia and the liberation of free nitrogen. More narrowly it includes only the latter phase, the liberation of free nitrogen by microörganisms acting on nitrates or nitrites. It is with this latter phase that my experiments were concerned. The importance for agriculture of denitrification in the soil has been greatly exaggerated. It is important if large amounts of fresh manure are added to soil rich in nitrates, but not otherwise.

Denitrification is carried out by a variety of microörganisms, chief among which are *B. denitrificans*, *B. pyocyaneus*, *B. fluorescens-liquefaciens*, and *B. Hartlebii*. Lipman (1902) found from 1.3 per cent to 25.6 per cent loss in nitrogen with pure cultures and as high as 35 per cent loss with mixed cultures. He also states that denitrifying organisms are found in all soils. These denitrifying organisms live in the presence of air but may live anaerobically.

Koch and Pettit (1910) found that denitrification varies with the moisture present. With an increase in moisture there is an increase in denitrification, and as the moisture is increased under laboratory conditions there is an increase in the nitrogen lost.

Jensen (1909) has pointed out that denitrification is always accompanied by oxidative processes.

My study of denitrification was carried out both in soils and in solutions. For the solution work the following medium was used—Giltay and Aberson's solution:

H_2O..........................	1000.00	K_2HPO_4.......................	2.00
KNO_3.........................	2.00	$CaCl$..........................	0.20
$MgSO_4$........................	2.00	Na_2CO_3.......................	4.25
Citric acid..................	5.00		

For the soil work, 100 grams of soil were inoculated with 0.2 gram of KNO_3. Anaerobic conditions were again obtained with sterile paraffin oil for the solution work, and by the absorption of oxygen with pyrogallic acid and caustic soda for the soil work. In the following experiment 200 cc. of Giltay and Aberson's solution were inoculated with 2 grams of the soils of the three types and then incubated for twenty-one days at 30° C. under aerobic and anaerobic conditions.

Experiment XIV. Denitrification in solution. Nitrogen in milligrams per 200 cc. of solution.

Aerobic

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN OR LOSS
Greenhouse....................	20.10	56.19	−36.09
Loam..........................	22.47	52.19	−29.72
Clay..........................	18.84	51.82	−32.98

Anaerobic

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN OR LOSS
Greenhouse....................	20.10	56.19	−36.09
Loam..........................	16.82	52.19	−35.37
Clay..........................	16.74	51.82	−35.08

Denitrification seems to proceed better under anaerobic conditions than under aerobic conditions. The difference is not very marked, though noticeable. Again the process seems to proceed best in the richest soil, probably due to the fact that there are more bacteria of the denitrifying type present.

In the following experiment washed air was bubbled through 200 cc. of the solution for twenty-one days at 30°C.

Experiment XV. Denitrification in solution. Nitrogen in milligrams per 200 cc. of solution.

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN OR LOSS
Greenhouse.....................	20.34	56.19	−35.85
Loam.........................	24.33	52.19	−27.86
Clay..........................	20.48	51.82	−30.34

Bubbling air through the solution does not inhibit the liberation of free nitrogen into the air by bacteria to any marked extent. In every case there is less nitrogen lost when air is bubbled through than under ordinary aerobic and anaerobic conditions, but there is not enough difference to be of any marked importance.

Loss of nitrogen under different conditions. Nitrogen in milligrams per 200 cc. of solution.

SOIL	EXCESS AIR	AIR	WITHOUT AIR
Greenhouse.....................	−35.85	−36.09	−36.09
Loam.........................	−27.86	−29.72	−35.37
Clay..........................	−30.34	−32.98	−35.08

From a study of these figures it is evident in each case, with each type of soil, that there is least nitrogen lost under excess air conditions and most lost under anaerobic conditions.

In the following experiment denitrification was carried on in solution (100 cc. of solution and 2 grams of soil) under anaerobic conditions, in the anaerobic apparatus (pyrogallic acid and caustic soda) along with a nitrogen fixation experiment.

Experiment XVI. Denitrification in solution. Nitrogen in milligrams per 100 cc. solution.

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN OR LOSS
Greenhouse.....................	11.76	28.10	−16.34
Loam.........................	12.88	26.09	−13.11
Clay..........................	16.24	25.91	− 9.67

Most denitrification goes on in the greenhouse soil and least in the clay.

Some further experiments were carried out with soil as a medium. One hundred grams of soil were inoculated with 10 cc. of a 2 per cent solution of KNO_3, and incubated twenty-one days at 30°C. under aerobic and anaerobic conditions.

Experiment XVII. Denitrification in soils. Nitrogen in milligrams per 10 grams of soil.

Aerobic

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN OR LOSS
Greenhouse......................	25.58	29.09	−3.51
Loam.........................	12.59	12.15	0.44
Clay...........................	10.57	7.11	3.46

Anaerobic

SOIL	NITROGEN AT END	NITROGEN AT BEGINNING	GAIN OR LOSS
Greenhouse......................	18.05	29.09	−11.04
Loam.........................	13.05	12.15	0.90
Clay...........................	11.31	7.11	4.20

With the greenhouse type of soil, denitrification took place, to a greater extent under anaerobic than under aerobic conditions. With the other two types of soil there was no loss of nitrogen. It is evident that soil as a medium does not give as good results as the solutions in regard to denitrification.

In general, denitrification goes on under aerobic and anaerobic conditions. An excess of air does not seriously inhibit the production of nitrogen, although slightly less nitrogen is lost. The process proceeds slightly better under anaerobic conditions, although almost as much nitrogen is lost under aerobic conditions. Denitrification proceeds better in solution than in soils, nitrogen being lost only in the greenhouse type of soil and not in the other two types.

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THE PREPARATION OF CULTURE MEDIA FROM WHOLE BLOOD

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Several European workers (Szasz, 1915 a, 1915 b; Schmitz, 1916; Lichtenstein, 1916), have called attention to the fact that it is possible to prepare from whole blood, a very satisfactory medium for the propagation of bacteria, which while possessing in some respects, advantages over media prepared from meat infusions, can be produced at a cost very much less. It was found that organisms which grew only sparsely or not at all on the ordinary culture media, would in most instances grow luxuriantly on the medium prepared from blood.

Comprehending the economic importance of such a method, and the advantage of being able to obtain a medium favoring the growth of obstinate organisms, I have prepared and tested several lots of this product with very pleasing results. The method employed is as follows:

Fresh beef blood is obtained 'in a clean container (a covered enamel bucket is a good receptacle) and allowed to clot. After allowing it to remain in the refrigerator several hours to give the serum a chance to separate, the clot is removed and finely ground in a meat chopping machine. This ground material is then replaced in the serum and the whole weighed. Two volumes of distilled water are then added and the mixture placed in an enamel pot and slowly brought to a boil, stirring continually to prevent burning. After boiling gently for five minutes the fluid portion is filtered off through cheesecloth and the residue put through a fruit press to extract as much more fluid as possible, using a towel or other heavy material to line

the inside of the press in order that the pulp may not be pressed through. The residue is then discarded and the fluid placed over the flame and again brought to a boil, the coagulated protein being skimmed off as it collects on the surface. A sufficient amount of concentrated acetic acid (approximately 0.5 cc. per liter of fluid) to cause flocculation is added, and the boiling continued for five minutes. The product is now ready to be filtered. For this purpose a stand is arranged holding three funnels, placed one above the other, the first containing absorbent cotton and the other two filter paper, and the material is filtered. The volume is then ascertained and 1 per cent peptone and 0.5 per cent sodium chloride are added, heating sufficiently to effect solution. The medium can now be titrated and the reaction corrected by neutralizing with sodium hydroxide, or if a solid medium is to be prepared the usual amount of agar may be added before titration. Sterilization is accomplished by autoclaving for one-half hour under 12 pounds pressure.

While the clot and the serum may be handled separately, i.e., by removing the clot, cutting it up and boiling with distilled water, filtering, then adding the serum and again boiling, this is not essential, as equally good results are obtained by handling the clot and serum together.

In the use of the bouillon alone I have found that the addition of a slight amount of carbohydrate (0.25 per cent glucose) favors the growth of some organisms is this medium. This is undoubtedly due to the fact that bouillon prepared from the blood is almost sugar-free, containing only a few hundredths of 1 per cent sugar. When agar is added the addition of carbohydrate is unnecessary.

The nitrogen content is also somewhat less in the blood medium than in that prepared from beef.

Several tests were undertaken to ascertain the comparative value of this medium and that prepared from beef. Tubes of bouillon, plain and glycerin agar were prepared from the blood bouillon and the same from beef bouillon and inoculated with various types of organisms, including streptococci, staphylococci,

B. typhi, B. bipolaris-septicus, B. diphtheriae, B. anthracis, B. tuberculosis (isolated from a bear), *B. abortus, B. pyocyaneus, B. mallei,* fungi, saccharomyces, etc. In all instances where there was a noticeable difference in growth on the two types of media, it was in favor of the blood preparation, such organisms as streptococci and *B. diphtheriae* growing with special luxuriance.

A test conducted on a lot of the medium prepared without the addition of peptone demonstrated the fact that it is possible to grow organisms such as streptococci, etc., on the peptone free medium but not so luxuriantly as when peptone is added.

In most instances the addition of glycerin is unnecessary; organisms which are usually grown on glycerin-agar, will, as a rule, grow very satisfactorily on plain agar prepared from the blood bouillon.

This medium, as can be seen from the method outlined above, is very easy to prepare, in fact more simple of preparation than the ordinary beef infusion, as the time required for trimming away fat, fascia, etc., is eliminated. This fact, together with the small cost and the adaptability of the medium for organisms which do not thrive well on the ordinary culture media should recommend it as a valuable product in the laboratory. It can practically take the place of serum-agar which is somewhat more difficult to prepare.

An effort was made to produce a concentrated extract from the blood, similar to beef extract. By considerably reducing the amount of distilled water added to the cut up blood clot and serum, a much more concentrated extract is obtained and this can be reduced to the desired consistency through evaporation over a water bath. Media prepared from this concentrated extract proved satisfactory in every way.

A concentrated extract from blood can also no doubt be prepared by the method used in packing houses for the production of meat extracts, i.e., through the use of heat and vacuum.

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PRELIMINARY NOTE ON THE CLASSIFICATION OF SOME LACTOSE FERMENTING BACTERIA

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The key given below is the result of a study of 333 lactose fermenting organisms isolated from soil, sewage, and various animal sources, including man, the horse, the sheep, the cow, and the pig.

The fermentation reactions were determined in peptone water containing the test substance.

Motility was observed in a soft agar medium (nutrient broth with 0.5 per cent agar) after six, and twenty-four, hours incubation at the body temperature. Six hours seems sufficient for differentiation.

Gelatin liquefaction was recorded for five weeks.

The methyl red and Voges-Proskauer reactions were determined in 0.5 per cent glucose-peptone-dipotassium phosphate solution.

It will be observed that the subdivisions are not based upon single characters, but upon differences in groups of characters. Where inspection is impractical, or insufficient to show which character is best correlated with others, considerable information may be obtained from a study of the coefficients of correlation. That character which gives the highest coefficient of correlation with the greatest number of characters studied is the best for classification, if subdivision is to be made entirely upon correlated characters.

It should perhaps be noted that the names assigned to the species in the key are tentative, and may be changed, if upon further study of the literature, they are found to be invalid.

A small group of organisms which resemble *B. aerogenes* with respect to gas formation from various carbohydrates etc., did not give the Voges-Proskauer reaction, and were neutral to methyl red after three days incubation at the body temperature. It has been previously observed that some organisms do not give the Voges-Proskauer reaction, and are not alkaline to methyl red until the fifth or seventh day of incubation. These organisms resemble very closely the *B. gasoformans non-liquefaciens* described by MacConkey (1909) who records the Voges-Proskauer reaction as positive or negative. As this name is a trinomial, and consequently invalid, the group is included, for the present, under *B. aerogenes*.

Key to the more common species of aerobic or facultative non-spore-forming bacteria which ferment lactose with gas formation

A. Voges-Proskauer reaction negative, usually acid (at least not alkaline) to methyl red, with no reversion on long standing; indol usually positive; polysaccharids (starch, dextrin, and inulin) negative.

Coli group.

    I. Sucrose positive.
        a. Motile; dulcitol, glycerol, and salicin usually positive.

                1. *B. communior.*

        b. Non-motile:
            1. Salicin positive; dulcitol and glycerol usually positive.

                2. *B. neapolitanus.*

            2. Salicin and dulcitol negative, glycerol usually negative.

                3. *B. coscoroba.*

    II. Sucrose negative.
        a. Salicin positive, dulcitol and glycerol usually positive.

                4. *B. coli.*
            1. Motile   *var. communis.*
            2. Non-motile.   *var. immobilis.*
        b. Salicin negative; dulcitol usually negative.
            1. Motile; glycerol usually positive.

                5. *B. Gruenthal.*

            2. Non-motile; glycerol usually negative.

                6. *B. acidi-lactici.*

B. Voges-Proskauer reaction positive; (occasionally only after long incubation); reaction to methyl red alkaline, or if acid at first, it reverts to a distinct alkaline reaction after long incubation (7 days); indol, usually negative; polysaccharids, starch inulin and dextrin, negative or positive.

Aerogenes-cloacae group.

I. Non-motile; gelatin rarely liquefied; indol, dulcitol and inulin negative or positive; sucrose, raffinose, mannitol, glycerol, salicin, dextrin, and starch positive.

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7. *B. aerogenes.*

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II. Motile; gelatin liquefied (often very slowly); indol, dulcitol, glycerol, inulin and starch usually negative (rarely positive); dextrin occasionally positive; sucrose, raffinose, salicin, and mannitol positive (rarely negative).

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8. *B. cloacae.*

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A NEW ICE SAMPLER

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There is much apparent dissatisfaction with the ordinary methods of sampling ice. This laboratory is now using an ice sampler based on the principle of a coal sampler. A brass cylinder, with the dimensions given in the diagram, has teeth cut in the lower end. These teeth are given a set. The apparatus fits into a carpenter's brace, which makes it possible to
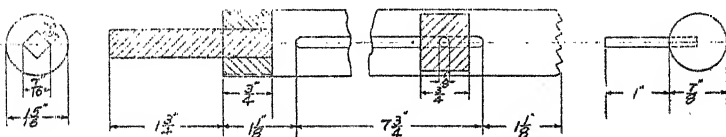
bore through a cake of ice with ease. The sample is not a solid core but consists of small chips of ice which are easily pushed out by means of a movable plug on the inside of the brass cylinder. It is necessary to flame the tube a little before the sample will slip out easily. With this apparatus, it is possible to get a representative sample of ice without contamination.

APPARENT RECOVERY OF A HEN INFECTED WITH BACILLARY WHITE DIARRHEA

As Determined by the Macroscopic Agglutination Test

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The following note deals with the case of a Bantam hen which at one time (1914), gave a positive agglutination test and at a later date (1916) gave a negative reaction.

The method of testing was essentially that devised by Jones. A polyvalent test fluid with proper controls was used.

The Bantam was one of a flock of several hundred birds of which approximately 65 per cent were infected. The bird was tested first in December, 1914; the reaction was very marked within 24 hours. In the fall of 1915 the test was applied again; the reaction was faint after 72 hours. During 1916 two tests were made, both of which were negative even after 72 hours.

That reacting fowls may lay eggs the yolks of which harbor the infective organism, *B. pullorum*, has been demonstrated by Rettger, Gage and others. The infection in this case was undoubtedly of ovarian origin because of the finding and isolation of pure cultures of *B. pullorum* (the infective agent in the bacillary form of white diarrhea) from two unhatched eggs of a sitting of eggs laid by this Bantam in 1915.

In view of the above observation however, we may ask,—is it not possible for an infected fowl to free herself of infection? or, is she once and for all time a bacillus carrier?

Although this single instance furnishes but limited evidence it suggests the possibility of recovery, or the throwing off of ovarian infection.

Further investigation is necessary to establish this point; and experiments are now in progress at this Station.

It is not recommended as good practice, to hold over infected stock in the hope of such a "recovery," as that indicated by these observations, because of the great risk from infected eggs.

OBSERVATIONS SUR L'INFLUENCE CHIMIQUE DES MILIEUX DE CULTURE SUR LE DÉVELOPPEMENT ET LA PRODUCTION DE L'INDOL PAR LES COLI-BACILLES ET PAR LES BACILLES TYPHIQUES

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I. INTRODUCTION

Les travaux consacrés à la recherche de milieux chimiquement définis, aptes au développement de tel ou tel microorganisme, sont fort nombreux. Il serait fastidieux de les passer en revue. Bornons nous à rappeler les très intéressants mémoires d'Armand Delille, André Mayer, G. Schaeffer et E. F. Terroine (1913), de Frouin (1912), de Galimard et Lacomme (1907), de Franzen Hartwich (1914), de Proskauer et Beck (1894), de Santon (1912) de Seliber (1914), de Tiffeneau et Marie (1912), de Trillat et Fouassier (1912), choisis parmi bien d'autres d'une tout aussi grande valeur.

Galimard et Lacomme sont partis d'une solution aqueuse fondamentale renfermant 1.5% de glycérine et 1.5% de constante minérale de Lepierre (chlorure de sodium 0.5%, sulfate de magnésie 0.05%, glycérophosphate de chaux 0.2%, bicarbonate de potasse en quantité suffisante pour neutraliser). Ils sont parvenus à cultiver les colibacilles dans ce milieu additionné de 1% soit de glycocolle, de leucine ou d'arginine, soit de 1% de tyrosine. Par contre ces microorganismes n'ont pas poussé dans le milieu fondamental précédent, auquel on a ajouté 1% d'acide asfaltique, de phénylalanine, de lysine ou d'urée. Une seule source d'azote suffit donc au développement des colibacilles. Ces microorganismes sont néanmoins plus difficiles dans le choix de leur nourriture azotée que les bacilles pyocyaniques qui ont

poussé en présence de chacun des acides aminés étudiés, à l'exception de la phénylalanine. Mais d'autre part les colibacilles sont bien moins exigeants que les bacilles paratyphiques et les vibrions du choléra qui n'ont poussé qu'en présence d'arginine ou de tyrosine et surtout que les staphylocoques pyogènes orangés qui ne se sont développés qu'en présence d'arginine. Quant aux bacilles typhiques, ils n'ont poussé en présence d'aucun des acides aminés précités, additionné seul à la solution aqueuse de glycérine et de constante minérale de Lepierre.

Galimard et Lacomme ont préparé, en partant de leur solution fondamentale, onze milieux renfermant chacun au moins deux acides aminés. En voici la composition: le premier milieu contient 0.75% de leucine, 0.2% d'alanine et des traces de tyrosine; le second milieu contient 1.5% d'un mélange d'acides monoaminés, de lysine et d'ornithine; le troisième milieu contient 0.3% de glycocolle, 0.05% de leucine, 0.01% de tyrosine et 0.1% d'acide aspartique; le quatrième milieu contient 0.45% de glycocolle, 0.01% de leucine, 0.05% de tyrosine et 0.15% de chlorhydrate d'arginine; le cinquième milieu contient 0.7% de glycocolle et 0.1% de chlorhydrate d'arginine; le sixième milieu contient 0.45% de leucine, 0.05% de tyrosine et 0.1% de chlorhydrate d'arginine; le septième milieu contient 0.2% de glycocolle, 0.4% de chlorhydrate d'arginine et 0.1% de chlorhydrate de lysine; le huitième milieu contient 0.1% de glycocolle et 0.9% de tyrosine; le neuvième milieu contient 0.5% de glycocolle et 0.5% d'urée; le dixième milieu contient 0.9% d'urée et 0.1% de chlorhydrate d'arginine; le onzième milieu contient 0.9% de glycocolle et 0.1% de chlorhydrate d'arginine.

Les bacilles pyocyaniques ont poussé dans ces onze milieux. Les colibacilles se sont développés partout à l'exception du second milieu. Le développement des bacilles paratyphiques n'a pas eu lieu dans les premier et second milieux, mais bien dans tous les autres. Il en a été de même des vibrions du choléra, mais ceux-ci n'ont, en outre, pas poussé dans le huitième milieu. Les staphylocoques pyogènes orangés ne se sont développés que dans les deux premiers milieux. Galimard et Lacomme ne sont point parvenus à cultiver les bacilles d'Eberth dans aucun des onze milieux.

Dans leurs importantes recherches, Galimard et Lacomme ont encore étudié la croissance d'autres espèces de microorganismes. Ils n'ont pas tenté d'évaluer le degré de développement des cultures de colibacilles ou des autres bactéries étudiées dans les divers milieux qu'ils ont employés. Ils n'ont pas non plus procédé à des tentatives de réensemencement sur un milieu solide, tel que l'agar par exemple, après un séjour plus ou moins long à l'étuve des divers milieux chimiquement définis ensemencés au moyen de colibacilles ou d'autres microorganismes.

Passons à un autre ordre de faits. On admet depuis longtemps déjà que le colibacille donne naissance d'une façon à peu près constante à de l'indol dans les cultures en milieu peptoné, tandis que le bacille typhique n'en produit pas dans ces conditions (Kitasato 1889). On s'est demandé aux dépens de quelle substance contenue dans les mélanges commerciaux de produits de désintégration des protéines, dénommés à tort "peptones," le colibacille donne la réaction de l'indol. On sait actuellement que c'est aux dépens du tryptophane. L'intensité de la réaction dépend de la teneur du milieu en ce composé. Il suffit de la présence de 0.03% de tryptophane dans une solution aqueuse renfermant, en outre, du sucre, de la glycérine, des phosphates de potasse (0.5%) et de magnésie (0.03%) et du lactate d'ammoniaque (0.5%) pour obtenir une réaction de l'indol très nette après ensemencement par des colibacilles et séjour de 24 a 48 heures à l'étuve (Zipfel 1913).

Les résultats des expériences de Galimard et Lacomme et de celles de Zipfel nous ont engagé à poursuivre des essais de culture des colibacilles et des bacilles typhiques dans des milieux chimiquement définis, dans l'espoir d'en trouver de particulièrement appropriés à la différenciation de ces deux espèces de microorganismes au moyen de la réaction de l'indol. Dans ce but, il y avait lieu de remplacer la "peptone de Witte" ou tout autre mélange de ce genre utilisé, en sus du bouillon ou mieux de l'eau physiologique (Escherich 1903) (Besson 1904) pour la recherche de la réaction de l'indol, par des protéoses, des peptides, des acides aminés, de l'urée, des corps puriques et pyrimidiques, des substances extractives. On peut ainsi ajouter,

soit au bouillon, soit à l'eau physiologique, de nombreux produits azotés en employant un seul ou plusieurs d'entre eux.

Le bouillon renferme, outre ses constituants salins et azotés, des substances ternaires tels que le glucose et l'inosite.   Il était, par conséquent, indiqué de se préoccuper de l'influence de ces corps et d'autres hydrates de carbone sur le développement des bacilles d'Eberth et des colibacilles et sur la réaction de l'indol.

C'est ainsi que nous avons été amené à entamer les recherches exposées ci-dessous.

Nous avons aussi consacré quelques expériences aux bacilles paratyphiques A et B, aux vibrions du choléra, aux bacilles pyocyaniques, aux staphylocoques pyogènes dorés, aux bacilles de l'entérite de Gärtner.

II. TECHNIQUE

Nous sommes partis de quatre milieux de culture: (1) le bouillon de boeuf;[1] (2) l'eau physiologique, c'est-à-dire une solution a 0.5% de chlorure de sodium chimiquement pur; (3) l'eau physiologique aminée, c'est-à-dire une solution aqueuse renfermant 0.5% de chlorure de sodium et 0.2% de glycyltryptophane; (4) l'eau peptonée, c'est-à-dire une solution aqueuse contenant 0.5% de chlorure de sodium et 1% de peptone de Witte.

On a réparti aseptiquement les divers milieux, tels quels ou additionnés d'un ou de plusieurs produits, par portions de 10 centimètres cubes, dans des tubes à essai steriles, puis on a de nouveau stérilisé ces tubes.

Pour chaque substance ou groupe de substances utilisé, ajouté a l'un ou à l'autre de ces milieux, on a eu soin de préparer deux tubes lors de chaque essai effectué avec la même espèce de microbes.

Dans chaque expérience, on s'est servi pour l'ensemencement de la même culture sur agar de colibacilles, de bacilles d'Eberth ou d'autres microorganismes.   Ces cultures nous ont été aimablement fournies par Messieurs les Professeurs Bordet et Gengou. On a eu soin de les rajeunir par réensemencement de façon a toujours partir de cultures poussant très vite en bouillon peptoné.

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[1] Préparé d'après la formule décrite dans le Manuel de bactériologie clinique de M. Furck, Bruxelles, 1910, p. 12.

Lors de chaque expérience, on a examiné une première série de tubes à essai au bout de 15 à 48 heures afin d'évaluer le degré de croissance en se basant sur les propriétés optiques (opalescence, trouble, voile, etc.) et sur l'examen microscopique. On a ensuite recherché la réaction de l'indol au moyen de l'aldéhyde paradiméthylaminobenzoïque, de la façon préconisée par Haenen (1905). On a parfois procédé à deux reprises à l'examen des propriétés optiques de cette série de tubes, en ayant naturellement soin, lors du premier examen, de maintenir les tubes bien bouchés de manière à éviter toute contamination.

On a examiné, en prenant ces précautions, à deux ou trois reprises, le degré de développement des diverses cultures dans la seconde série de tubes à essai. On a prélevé une oese de chaque milieu liquide, lors de l'un de ces examens, et on a réensemencé sur agar. On a examiné à une ou deux reprises le degré de croissance de ces cultures sur agar. Après le dernier examen des propriétés optiques des cultures en bouillon ou en eau physiologique (aminée ou non), on a procédé à la recherche de la réaction de l'indol.

Monsieur le Professeur Kossel (d'Heidelberg) nous a aimablement fourni le sulfate de clupéine. Monsieur le Professeur Emil Fischer (de Berlin) a eu la grande obligeance de nous envoyer de la glycylglycine, de la diglycylglycine et de l'alanylglycylglycine.[2] Nous avons préparé les protéoses d'après la méthode de E. P. Pick (1899), la leucine et la tyrosine de la manière habituelle. Les autres produits provenaient des maisons Guibler (de Dresde et Leipzig), Kalle (de Biebrich) et Kahlbaum (de Berlin).

III. EXPÉRIENCES

Nous avons choisi les expériences les plus démonstratives pour en reproduire ci-dessous les résultats sous forme de tableaux.

Le plus ou moins grand degré de croissance des cultures dans les divers milieux expérimentés, évalué de la façon indiquée ci-dessous, a été exprimé dans ces tableaux, par un nombre plus

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[2] Nous tenons à remercier bien vivement Messieurs les Professeurs Bordet, Emil Fischer, Gengou, et Kossel de leur extrême obligeance.

ou moins considérable de signes +. Lorsqu'on n'a pu déceler aucun développement microbien dans un tube, on trouvera en regard le signe −. Il est parfois arrivé de constater une légère opacité ou un léger trouble du milieu, sans qu'on ait observé de microbes à l'examen microscopique de préparations colorées provenant du tube en question et sans qu'on soit parvenu a déceler de développement des microorganismes après réensemencement sur agar. Ces cas douteux ont été mentionnés par le signe +?

Pour ce qui concerne la réaction de l'indol, les signes + et − veulent dire que la réaction a été positive ou negative. Dans certains cas, la réaction a été douteuse. On n'est parfois pas parvenu a déceler de coloration rose de la culture de colibacilles lors de l'addition de l'aldéhyde paradiméthylaminobenzoïque et de l'acide chlorhydrique, mais après agitation avec le chloroforme ou l'alcool amylique, le solvant a pris une légère teinte rose. Nous avons mentionné, dans les tableaux V et VI, ces cas par le signe +?. D'autrefois, on a observé l'apparition d'une coloration rose lors de l'addition à la culture étudiée d'acide chlorhydrique et d'aldéhyde paradiméthylaminobenzoïque, sans que cette coloration passe dans le chloroforme ou l'alcool amylique. On doit considérer ces cas, dont les tableaux V et VI offrent des exemples, comme très probablement négatifs; nous les avons néanmoins, par plus de prudence, qualifiés de douteux en leur attribuant le signe +?.

A. *Expériences effectuées seulement avec des colibacilles et des bacilles typhiques*

1. *Expériences effectuées en partant du bouillon comme milieu de culture.* A titre d'exemple, nous reproduisons, dans le tableau I, l'expérience la plus démonstrative.

La croissance des colibacilles a eu lieu dans les divers milieux expérimentés, bien qu'à un moindre degré que dans le bouillon additionné de 1% de peptone de Witte. Le développement a été le plus rapide en présence d'hétéroalbumose, de glycyltryptophane, de leucine et de gliadine, le plus lent en présence d'alanylglycylglycine, de glycocolle, d'alanine.

On n'a observé la réaction de l'indol que dans la culture de colibacilles en bouillon additionné de 0.3% de glycyltryptophane. Au bout de 48 heures, la réaction de l'indol était, dans ce tube bien plus intense que dans le tube témoin de bouillon peptoné.

La croissance des bacilles typhiques a été plus intense en bouillon additionné de 0.5% de gliadine qu'en bouillon peptoné. Elle a été tout aussi abondante en bouillon additionné de 0.5% d'hétéroalbumose qu'en bouillon peptoné. Dans les autres milieux, les bacilles d'Eberth n'ont pas poussé ou très peu.

2. *Expériences effectuées en partant de l'eau physiologique comme milieu de culture.* On trouvera, dans les tableaux II à IV, les protocoles des trois expériences effectuées en partant de ce milieu.

Les colibacilles se sont développés, tout aussi bien qu'en présence de peptone de Witte, en présence de leucine et presque aussi bien en présence de protoalbumose, d'histidine, de diglycylglycine, de glycyltryptophane (seul ou additionné de saccharose), de caséine, de chlorhydrate de guanidine, de taurine, de diméthyldiphénylurée. Le développement a été quelque peu retardé en présence de thioalbumose, de glycocolle, d'alanine, de gliadine, de xanthine, d'acide glycocholique. Il a été notablement retardé dans les tubes renfermant de l'acide aspartique, de la tyrosine, de la phénylalanine, de l'acide urique, de l'acide cyanurique, de la sarcosine, de la créatine. On n'a observé aucun développement dans les tubes contenant de l'hétéroalbumose, de l'acide glutanique, du phénylglycocolle, du sulfate de clupéine, de l'allantoïne, de l'hypoxanthine, de la caféine, de l'alloxane, de la bétaïne, de l'acide barbiturique, de l'acide hippurique, de l'acide glycocholique, de l'urée, du chlorhydrate de métaphénylenediamine.

On n'a constaté la réaction de l'indol que dans les cultures de colibacilles en eau physiologique, additionnée soit de peptone de Witte, soit de glycyltryptophane.

Les bacilles d'Eberth ont très bien poussé en eau peptonée. Leur développement a été encore plus abondant en eau physiologique additionnée de 0.3% de gliadine. Ils ont faiblement poussé dans la solution de chlorure de sodium à 0.5% renfermant 0.3% soit de caséine, soit de sarcosine, soit de taurine, soit de

TABLEAU I

COMPOSITION DES MILIEUX: BOUILLON RENFERMANT	ÉCHANTILLON EMPLOYÉ	COLIBACILLES						BACILLES TYPHIQUES					
		Première culture				Deuxième culture (réensemencement sur agar après 23 heures)		Première culture				Deuxième culture (réensemencement sur agar après 23 heures)	
		Degré de croissance après		Réaction de l'indol après		Degré de croissance après		Degré de croissance après		Réaction de l'indol après		Degré de croissance après	
		23 heures	48 heures	23 heures	48 heures	7 heures	30 heures	23 heures	48 heures	23 heures	48 heures	7 heures	30 heures
1% de peptone de Witte.	premier	++	+++	+	—	+	+	+?	+	—	—	+	++
	deuxième	++	+++	+	+	+	+	+?	+	—	—	+	++
0.05% d'heteroalbumose.	premier	+	++	—	—	+	+	+?	+	—	—	+	—
	deuxième	+?	++	—	—	+	+	+?	+	—	—	—	—
0.3% d'alanylglycylglycine.	premier	+?	+	+	—	+	+	+?	—	—	—	—	—
	deuxième	+	+	—	—	+	+	+?	—	—	—	—	—
0.3% de glycyltryptophane.	premier	+	++	—	++	+	+	+?	+?	—	—	+?	+?
	deuxième	+?	++	—	—	+	+	+?	+?	—	—	+?	+?
0.3% de glycocolle.	premier	+?	+	—	—	+	+	+?	—	—	—	+?	+?
	deuxième	+?	+	—	—	+	+	+?	—	—	—	+	+
0.3% d'alanine.	premier	+?	+	—	—	+	+	+?	—	—	—	+?	+
	deuxième	+	++	—	—	+	+	—	—	—	—	+	+
0.3% de leucine.	premier	+	+	—	—	+	+	+?	—	—	—	—	—
	deuxième	+	++	—	—	+	+	+?	—	—	—	—	—
De la tyrosine (solution saturée).	premier	+	+	—	—	+	+	+?	—	—	—	+	+
	deuxième	+	+	—	—	+	+	+?	—	—	—	+?	+?
0.3% de phénylalanine.	premier	+	+	—	—	+	+	+?	—	—	—	—	—
	deuxième	+?	+	—	—	+	+	+?	—	—	—	—	—
0.3% d'acide urique.	premier	+	+	—	—	+	+	+?	—	—	—	+	+
	deuxième	+	+	—	—	+	+	+	—	—	—	—	—
0.5% de gliadine.	premier	+	++	—	—	+	+	+	+	—	—	++	++
	deuxième	+	++	—	—	+	+	+	+	—	—	++	++

xanthine. Dans tous les autres milieux, nous n'avons pas constaté la moindre croissance des bacilles d'Eberth.

Nous confirmons donc les résultats negatifs obtenus par Galimard et Lacomme avec les bacilles typhiques en présence de glycocolle, de leucine, de tyrosine, d'acide aspartique, de phénylalanine et d'urée comme source unique d'azote, les résultats positifs obtenus par ces auteurs avec les colibacilles en présence de glycocolle, de leucine et de tyrosine ainsi que l'absence de développement de cette dernière espèce de microorganismes dans un milieu renfermant seulement de l'urée comme composé azoté. Par contre, grâce à la méthode de réensemencement sur agar, nous avons pu mettre en évidence que l'acide aspartique et la phénylalanine n'étaient pas dénués de toute valeur nutritive pour les colibacilles.

3. *Expériences effectueés en partant de l'eau physiologique aminée comme milieu de culture.* Nous avons remarqué, au cours des expériences effectuées en se servant soit du bouillon, soit de l'eau physiologique comme base des milieux de culture que l'addition de glycyltryptophane à ces milieux permet aux colibacilles un rapide et intense développement d'indol. Il nous a semblé intéressant de rechercher l'influence exercée sur ce phénomène par l'addition de diverses substances contenues dans le bouillon. Dans ce but, nous avons preparé une solution aqueuse renfermant 0.5% de chlorure de sodium et 0.2% de glycyltryptophane. C'est à ce milieu de culture que nous avons donné, comme nous l'avons, du reste, déjà indiqué plus haut, le nom d'eau physiologique aminée. On trouvera, dans le tableau V, le protocole d'une expérience de ce genre.

Des divers produits examinés, seul la glucose a accru le développement des colibacilles en eau physiologique aminée. La taurine, l'acide barbiturique, la créatine, la sarcosine, l'urée ont légèrement entravé la croissance de ces microorganismes. L'acide cyanurique, la guanidine, la bétaïne et la xanthine l'ont davantage entravée. L'inosite l'a retardée de façon considérable. Quant à la caféine et à l'allantoine, elles paraissent l'avoir totalement empêchée.

TABLEAU II

COMPOSITION DES MILIEUX: EAU PHYSIOLOGIQUE À 0.5% RENFERMANT:	ÉCHANTILLON EMPLOYÉ	COLIBACILLES Première culture — Degré de croissance après			Réaction de l'indol après		Deuxième culture (réensemencement sur agar après 24 heures): Degré de croissance après 15 heures	BACILLES TYPHIQUES Première culture — Degré de croissance après			Réaction de l'indol après		Deuxième culture (réensemencement sur agar après 24 heures): Degré de croissance après 15 heures	REMARQUES
		15 heures	36 heures	50 heures	15 heures	50 heures		15 heures	36 heures	50 heures	15 heures	50 heures		
1% peptone de Witte	premier	+	+	+	+	+	+	+	+	+	−	−	+	Les cultures de colibacilles présentent, après 50 heures, une couleur rosée.
	deuxième	+						+						
0.5% d'hétéroalbumose	premier	+?	+?	+?	−	−	−	+?	+?	+?	−	−	−	
	deuxième	+?						+?						
0.5% de protoalbumose	premier	+	+	+	−	−	+	+?	+?	+?	−	−	−	
	deuxième	+						+?						
0.3% de thioalbumose	premier	+?	+	+	−	−	+	+?	+?	+?	−	−	−	
	deuxième	+?						+?						
0.3% de diglycylglycine	premier	+	+	+	−	−	+	−	−	−	−	−	−	
	deuxième	+						−						
0.3% de glycyltryptophane	premier	+	+	+	+	+	+	+?	+?	+?	−	−	−	
	deuxième	+						+?						
0.3% de glycyltryptophane et 0.5% de saccharose	premier	+	+	+	−	+	+	−	−	−	−	−	−	
	deuxième	+						−						

		1	2	3	4	5	6	7	8	9	10	L'acidité du milieu pourrait bien être la cause de l'inhibition de la croissance
0.3% de glycocolle..	premier	+?	+	+	—	—	—	—	—	—	—	
	deuxième	+?	+	+	—	—	—	—	—	—	—	
0.3% d'alanine......	premier	+?	+	+	—	—	—	—	—	—	—	
	deuxième	+?	+	+	—	—	—	—	—	—	—	
Acide aspartique (solution neutralisée)...	premier	+?	+?	+?	—	+?	—	—	—	—	—	
	deuxième	+?	+?	+?	—	—	—	—	—	—	—	
0.3% d'acide glutanique...	premier	+?	+?	+?	—	+?	—	—	—	—	—	
	deuxième	+?	+?	+?	—	—	—	—	—	—	—	
0.3% de leucine...	premier	+	++	++	—	+	—	+?	—	—	—	
	deuxième	+	++	++	—	+	—	+?	—	—	—	
Tyrosine (solution saturée)...	premier	+	+	+	—	+	—	—	—	—	—	
	deuxième	—	—	—	—	+	—	—	—	—	—	
0.3% de phenylalanine...	premier	+?	+?	+?	—	+	—	—	—	—	—	
	deuxième	+?	+?	+?	—	+	—	—	—	—	—	
0.3% d'acide urique.	premier	+?	+?	+?	—	+	—	—	—	—	—	
	deuxième	+?	+?	+?	—	+	—	—	—	—	—	

TABLEAU III

COMPOSITION DES MILIEUX: EAU PHYSIOLOGIQUE À 0.5% RENFERMANT 0.3%	ÉCHANTILLON EMPLOYÉ	COLIBACILLES					BACILLES TYPIQUES					
		Première culture				Deuxième culture (réensemencement sur agar après 48 heures): Degré de croissance après 24 heures	Première culture				Deuxième culture (réensemencement sur agar après 48 heures): Degré de croissance après 24 heures
		Degré de croissance après		Réaction de l'indol après			Degré de croissance après		Réaction de l'indol après		
		48 heures	96 heures	48 heures	96 heures		48 heures	96 heures	48 heures	96 heures	
De caséine.	premier	+	+	–	–	+	+?	+?	–	–	+
	deuxième	+					+?				
De gliadine.	premier	+?	+	–	–	+	++	++	–	–	+
	deuxième	+?					++				
De sulfate de clupéine.	premier	–	–	–	–	–	–	–	–	–	–
	deuxième	–					–				
De créatine.	premier	+?	+	–	–	–	–	–	–	–	–
	deuxième	+?					–				
De chlorhydrate de guanidine.	premier	+	+.	–	–	+	+?	–	–	–	+
	deuxième	+					+?				
D'allantoine.	premier	+?	+?	–	–	–	–	–	–	–	–
	deuxième	+?					–				
De xanthine.	premier	+?	+	–	–	+	–	+	–	–	+
	deuxième	+?					–				
D'hypoxanthine.	premier	+?	+?	–	–	–	–	+?	–	–	–
	deuxième	+?					–				
De caféine.	premier	+?	+?	–	–	–	–	–	–	–	–
	deuxième	+?					–				
D'acide hippurique.	premier	+?	+?	–	–	–	–	–	–	–	–
	deuxième	+?					–				

De taurine............	{ premier	+	+?	+	—	+	+?	+	—	—	+
	deuxième	+	+?	+	—	+	+?	+	—	—	—
D'urée...............	{ premier	+?	+?	—	—	—	+?	+?	—	—	—
	deuxième	++	+?	—	—	—	+?	+?	—	—	—
De dimethyldiphén-ylurée..............	{ premier	+	+	+	—	+	—	+?	—	—	—
	deuxième	+?	+	—	—	+	—	+?	—	—	—
D'acide glyocoholique.	{ premier	+?	+	—	—	+	—	+?	—	—	—
	deuxième	+?	+	—	—	+	—	+?	—	—	—
De chlorhydrate de métaphenylenediam-ine...............	{ premier	—	—	—	—	—	—	—	—	—	—
	deuxième	—	—	—	—	—	—	—	—	—	—

TABLEAU IV

COMPOSITION DES MILIEUX: EAU PHYSIOLOGIQUE À 0.5% RENFERMANT 0.3%	ÉCHANTILLON EMPLOYÉ	COLIBACILLES						BACILLES TYPIQUES					
		Première culture					Deuxième culture (réensemencement sur agar après 96 heures): Degré de croissance après 24 heures	Première culture					Deuxième culture (réensemencement sur agar après 96 heures): Degré de croissance après 24 heures
		Degré de croissance après			Réaction de l'indol après			Degré de croissance après			Réaction de l'indol après		
		24 heures	48 heures	96 heures	48 heures	96 heures		24 heures	48 heures	96 heures	48 heures	96 heures	
De phénylglycocolle......	premier	−?	−	+?	−	−	−	+?	+?	+?	−	−	−
	deuxième	−?	+		−	−		+?	+?		−	−	
D'histidine............	premier	+	+	+	−	−	+	−	−	−	−	−	−
	deuxième	+	+		−	−		−	−		−	−	
D'alloxane............	premier	+?	+?	+?	−	−	−	−	−	−	−	−	−
	deuxième	+?	+?		−	−		−	−		−	−	
D'acide barbiturique......	premier	+?	+?	+?	−	−	+	−	−	−	−	−	−
	deuxième	+?	+?		−	−		−	−		−	−	
D'acide cyanurique......	premier	+?	+?	+	−	−	+	+?	+?	+?	−	−	−
	deuxième	+?	+?		−	−		+?	+?	+?	−	−	
Sarcosine............	premier	−	−	+	−	−	+	−	−	−	−	−	+
	deuxième	−	−		−	−		−	−	+	−	−	
De bétaïne............	premier	+?	+?	+?	−	−	−	−	−	−	−	−	−
	deuxième	+?	+?		−	−		−	−		−	−	

TABLEAU V

COMPOSITION DES MILIEUX: EAU PHYSIOLOGIQUE RENFERMANT	ÉCHANTILLON EMPLOYÉ	COLIBACILLES				BACILLES TYPHIQUES			
		Degré de croissance après		Réaction de l'indol après		Degré de croissance après		Réaction de l'indol après	
		16 heures	48 heures	16 heures	48 heures	16 heures	48 heures	16 heures	48 heures
Rien d'autre.......	premier	+++		+		+?		—	
	deuxième	+++	+++		++	+?	+?		—
0.2% de taurine.....	premier	++		++		+?		—	
	deuxième	++	++		++	+?	+?		—
0.2% de caféine....	premier	+?		+?		+		—	
	deuxième	+?	+?		+?	+	+		—
0.2% d'allantoine..	premier	+?		—?		—		—	
	deuxième	+?	+?		—?	—	+?		—
0.2% d'alloxane....	premier	+?		—?		+?		—	
	deuxième	+?	+		—?	+?	+		—
0.2% d'acide cyanurique.............	premier	+		+		+?		—	
	deuxième	+	+		+	+?	—		—
0.2% d'acide barbiturique.........	premier	++		+		+		—	
	deuxième	++	++		+	+	+		—
0.2% de créatine...	premier	++		+?		+?		—	
	deuxième	++	++		+	+?	+?		—
0.2% de sarcosine...	premier	++		++		+		—	
	deuxième	++	++		++	+	+		—
0.2% de guanidine..	premier	+		+		+		—	
	deuxième	+	+		++	+	+		—
0.2% de bétaïne....	premier	+		+		+?		—	
	deuxième	+	+		+	+?	+?		—
0.2% de xanthine...	premier	+		+		+?		—	
	deuxième	+	+		+	+?	+		—
0.2% d'inosite......	premier	+?		+?		+?		—	
	deuxième	+?	+		+	+?	+?		—
0.2% d'urée........	premier	++		+		+?		—	
	deuxième	++	++		+	+	+		—
0.2% de glucose....	premier	++++		+?		—		—	
	deuxième	++++	++++		+	—	+?		—

La réaction de l'indol a été favorisée, pour les cultures de colibacilles en eau physiologique aminée, par la sarcosine et la taurine. Elle n'a pas été influencée par la guanidine. Son intensité a diminué dans les tubes renfermant 0.2% d'acide

cyanurique, d'acide barbiturique, de bétaïne ou d'urée et encore davantage dans les tubes contenant 0.2% de créatine, de glucose ou d'inosite. Dans ces trois derniers milieux, les tubes examinés au bout de 16 heures n'ont pas montré de coloration rosée lors de l'addition d'aldéhyde paradiméthylaminobenzoïque et d'acide chlorhydrique, bien qu'après agitation avec du chloroforme, celui-ci soit devenu légèrement rose. Nous n'oserions pas affirmer qu'on se trouve bien en présence de la réaction de l'indol dans ces cas. Il en a aussi été ainsi au bout de 48 heures pour les tubes renfermant 0.2% de caféine, dans lesquels on n'a, du reste, pas pu déceler de colibacilles. Nous n'avons pas non plus observé de réaction de l'indol dans les tubes renfermant de l'allantoine ou de l'alloxane, puisque la coloration rose apparue alors après l'addition de l'aldéhyde paradiméthylaminobenzoique et de l'acide chlorhydrique n'a pas passé dans le chloroforme.

Les bacilles d'Eberth n'ont poussé que dans l'eau physiologique aminée contenant de l'acide barbiturique, de la caféine, de la guanidine, de la sarcosine, de la xanthine ou de l'urée. Rappelons qu'ils ne se sont pas développés dans l'eau physiologique renfermant de l'acide barbiturique (tableau IV), de la caféine, du chlorhydrate de guanidine ou de l'urée (tableau III) sans glycyltryptophane. Ils ne se sont développés de façon appréciable qu'entre la 48ème et la 96ème heure dans l'eau physiologique additionnée seulement de xanthine (tableau III) ou de sarcosine (tableau IV). L'association du glycyltryptophane à la xanthine a permis aux bacilles d'Eberth de se développer abondamment entre la 16ème et la 48ème heure. Dans les tubes renfermant à la fois le glycyltryptophane et la sarcosine, les bacilles typhiques se sont même déjà développés avant la 16ème heure de séjour a l'étuve.

Ces resultats nous ont engagé à étudier les effets de l'association de plusieurs produits de glycyltryptophane sur la croissance des colibacilles et des bacilles d'Eberth et sur la réaction de l'indol dans les cultures de colibacilles. Comme l'on a déjà entrepris de multiples recherches relatives à l'addition de la glucose ou d'autres hydrates de carbone aux milieux destinés à cultiver

et surtout à différencier ces deux espèces de microorganismes, nous nous sommes beaucoup préoccupés de la présence de la glucose dans les milieux de culture. Nous donnons, dans le tableau VI, les résultats d'une série d'essais ainsi effectués avec des mélanges de diverses compositions.

On sait, par les résultats relatés dans le tableau V, que l'addition de la glucose à l'eau physiologique aminée favorise le développement des colibacilles, mais entrave la réaction de l'indol. L'addition de la sarcosine à l'eau physiologique aminée favorise la réaction de l'indol, mais entrave le développement des microorganismes. L'addition de la créatine à l'eau physiologique aminée diminue la rapidité de développement des colibacilles et l'intensité de la réaction de l'indol. Or, d'après le tableau VI, l'addition à l'eau physiologique aminée à la fois de créatine, de glucose et de sarcosine, dans les mêmes proportions que celles utilisées séparément dans les expériences du tableau V, a favorisé le développement des colibacilles et a accru, du moins dans un cas, l'intensité de la réaction de l'indol. On ne peut donc conclure aux effets d'un mélange de corps azotés sur la croissance des colibacilles et sur l'intensité de la réaction de l'indol dans leurs cultures en se basant sur les résultats obtenus avec ces corps examinés isolément.

La solution à 0.5% de chlorure de sodium renfermant 0.2% de glycyltryptophane, 0.2% de glucose, 0.2% de créatine et 0.2% de sarcosine a amené une abondante croissance des bacilles d'Eberth entre la 16ème et la 88 ème heure consecutives à l'ensemencement, alors qu'on a obtenu des résultats douteux ou négatifs dans les tubes témoins ne renfermant que du glycyltryptophane et dans ceux contenant du glycyltryptophane et de la glucose ou de la créatine. Les effets favorables paraissent ici être dûs à la présence de la sarcosine, qui amène en eau physiologique (tableau IV) un développement faible et tardif des bacilles d'Eberth et en eau physiologique aminée (tableau V) un développement plus précoce de ces microorganismes.

Si à la solution aqueuse renfermant 0.5% de chlorure de sodium, 0.2% de glycyltryptophane, 0.2% de glucose, 0.2% de créatine et 0.2% de sarcosine, l'on ajoute encore de la bétaïne

TABLEAU VI

COMPOSITION DES MILIEUX: EAU PHYSIOLOGIQUE AMINÉE RENFERMANT	ÉCHANTILLON EMPLOYÉ	COLIBACILLES — Première culture — Degré de croissance après 16 heures	88 heures	Réaction de l'indol après 16 heures	88 heures	Deuxième culture (réensemencement sur agar après 88 heures): Degré de croissance après 24 heures	BACILLES TYPHIQUES — Première culture — Degré de croissance après 16 heures	88 heures	Réaction de l'indol après 16 heures	88 heures	Deuxième culture (réensemencement sur agar après 88 heures): Degré de croissance après 24 heures
Rien d'autre	premier	++	++	+	+	++	+?	+?	—	—	—
	deuxième	++					+?				
Glucose 0.2% / + sarcosine 0.2% / + creatine 0.2%	premier	+	+++	+?	++	++	+?	++	—	—	++
	deuxième	+					+?				
Glucose 0.2% / + sarcosine 0.2% / + creatine 0.2% / + bétaïne 0.2% / + inosite 0.2%	premier	+?	+?	−?	−?	—	+?	+	—	—	—
	deuxième	+?					+?				
Glucose 0.2% / + sarcosine 0.2% / + creatine 0.2% / + bétaïne 0.2% / + guanidine 0.2%	premier	+?	+?		—	—	—	+?	—	—	+?
	deuxième	+?					—				
Glucose 0.2% / + sarcosine 0.2% / + créatine 0.2% / + bétaïne 0.2% / + xanthine 0.2%	premier	++	+++	+	++	++	++	+	—	—	+?
	deuxième	++					++				

Milieu		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Glucose 0.2% + sarcosine 0.2% + alloxane 0.2% + taurine 0.2%	premier	+	+++	+?	+	++	+	+?	−	−	−?
	deuxième	+					+?				
Glucose 0.2% + sarcosine 0.2% + alloxane 0.2% + taurine 0.2% + inosite 0.2%	premier	+	++	+	+?	+	+?	+?	−	−	−?
	deuxième	+?					+?				
Glucose 0.2% + sarcosine 0.2% + acide barbiturique 0.2% + acide cyanurique 0.2%	premier	+	++	+	+	++	+?	+?	−	−	−?
	deuxième	+					+?				
Histidine 0.2%	premier	+	++	++	++	++	+?	+	−	−	−?
	deuxième	+		+			−				
Histidine 0.2% + glucose 0.2%	premier	++	+++	+	+?	++	+?	+	−	−	+?
	deuxième	++					+				
Histidine 0.2% + glucose 0.2% + inosite 0.2%	premier	+?	+?	−	−	−	−	−	−	−	−
	deuxième	+?					−				

et de l'inosite, la croissance des colibacilles et la réaction de l'indol sont entravées ou même complètement empêchées, et ce n'est qu'au bout de 88 heures qu'on constate un faible développement des bacilles d'Eberth. Ceci s'explique aisément, car l'addition de bétaïne et surtout celle d'inosite à l'eau physiologique aminée entrave la croissance des colibacilles. La réaction de l'indol est plus intense dans les cultures de colibacilles en eau physiologique aminée telle quelle qu'en ce même milieu additionné de bétaïne. Elle est plus intense et plus précoce en eau physiologique aminée qu'en cette solution additionnée d'inosite.

Substituons la guanidine à l'inosite et partons d'une solution aqueuse renfermant 0.5% de chlorure de sodium, 0.2% de glycyltryptophane, 0.2% de glucose, 0.2% de créatine, 0.2% de sarcosine, 0.2% de bétaine et 0.2% de guanidine. Il ne se développe ni colibacilles ni bacilles d'Eberth, bien qu'on n'ait découvert aucune influence inhibitoire de l'association de la guanidine au glycyltryptophane sur la croissance des bacilles typhiques et que l'addition de guanidine à l'eau physiologique aminée ait, dans les essais relatés dans le tableau V, entravé mais nullement empêché le développement des colibacilles.

Remplaçons, dans le milieu dont nous venons de parler, la guanidine par la xanthine. Le développement des colibacilles s'effectue plus vite et la réaction de l'indol apparaît plus tôt que dans les tubes renfermant seulement du glycyltryptophane, de la glucose, de la créatine, et de la sarcosine. Et pourtant l'association de la xanthine au glycyltryptophane entrave, d'après le tableau V, le développement des colibacilles et diminue l'intensité de la réaction de l'indol dans leurs cultures.

Si les résultats précédents se confirment, la solution aqueuse renfermant 0.5% de chlorure de sodium, 0.2% de glycyltryptophane, 0.2% de glucose, 0.2% de créatine, 0.2% de sarcosine, 0.2% de bétaïne et 0.2% de xanthine, semble particulièrement appropriée à la recherche de la réaction de l'indol dans les cultures de colibacilles. Remarquons toutefois que, dans ce milieu, le développement des bacilles d'Eberth est déjà très net au bout de 16 heures. Il est vrai, qu'il ne paraît, plus s'accroître par la suite. L'effet favorable sur la culture des bacilles typhiques

est très probablement dû à l'association de la sarcosine et de la xanthine au glycyltryptophane, si l'on s'en rapporte aux données du tableau V.

Étudions maintenant un milieu formé de glycyltryptophane, de glucose, d'alloxane, de sarcosine et de taurine. Le développement des colibacilles est tout aussi intense et précoce que dans le milieu précédent. Par contre, la réaction de l'indol apparaît plus tard que dans l'eau physiologique aminée et n'est pas plus intense que dans celle-ci. Les bacilles d'Eberth se développement parfois déjà au bout de 16 heures dans ce milieu,—d'autres fois on ne peut les déceouvrir, même au bout de 88 heures.

Ajoutons de l'inosite au milieu dont nous venons de nous occuper. Ceci suffit à faire disparaître les bons effets sur le développement des colibacilles, signalés ci-dessus. La croissance de ces microorganismes est même légèrement entravée par rapport à l'eau physiologique aminée. Ceci dépend certes de l'inosite. En effet, d'après le tableau III, la taurine ne paraît pas, ou ne paraît guère influencer de façon défavorable le développement des colibacilles. Il est vrai que les tableaux IV and V tendent à démontrer une action inhibitoire de l'alloxane sur la croissance des colibacilles, mais elle n'apparaît point dans le mélange de glycyltryptophane, de glucose, de sarcosine, de taurine et d'alloxane. Par contre, les résultats obtenus dans ce milieu et les données des tableaux IV et V tendent à démontrer que l'action nocive de l'alloxane s'ajoute à celle de l'inosite pour empêcher la réaction de l'indol dans les cultures de colibacilles ou tout au moins retarder l'apparition et l'intensité de cette réaction.

D'après les données du tableau VI, les bacilles d'Eberth n'ont pas, ou n'ont guère poussé dans le milieu complexe (glycyltryptophane, glucose, sarcosine, alloxane, taurine, inosite) dont il vient d'être question.

L'addition de glucose, de sarcosine, d'acide barbiturique et d'acide cyanurique à l'eau physiologique aminée ne modifie en rien la croissance des colibacilles et la réaction de l'indol dans leurs cultures. Les bacilles d'Eberth ne semblent pas avoir davantage poussé dans ce milieu que dans l'eau physiologique aminée.

Si l'on se base sur les résultats consignés dans le tableau VI, on constate que l'association de l'histidine au glycyltryptophane a eu des effets heureux pour la croissance des colibacilles et des bacilles d'Eberth et pour l'intensité de la réaction de l'indol dans les cultures de colibacilles. Les bacilles typhiques n'ont toutefois commencé à se developper de façon appréciable qu'entre la $16^{ème}$ et la $88^{ème}$ heure consécutives à l'ensemencement, alors que les cultures de colibacilles ont déjà présenté très nettement la réaction de l'indol au bout de 16 heures. On se trouve donc de nouveau en présence d'un milieu specialement approprié à la différenciation des colibacilles d'avec les bacilles d'Eberth par la recherche de la réaction de l'indol.

Augmentons la complexité du milieu en y ajoutant de la glucose. Le développement des colibacilles et celui des bacilles d'Eberth sont plus favorisés qu'en l'absence de cet hydrate de carbone. Par contre. la réaction de l'indol est moins forte, et même parfois douteuse, dans les cultures de colibacilles. Ce milieu convient, par conséquent, beaucoup moins que le précédent à la recherche de la réaction de l'indol dans les cultures de colibacilles.

Ajoutons de l'inosite au glycyltryptophane, au glucose et à l'histidine. On n'observe plus aucun développement des bacilles d'Eberth et tel paraît être aussi le cas pour les colibacilles. L'influence défavorable de l'inosite ressort à nouveau des faits précédents.

4. *Comparaison de l'action de l'addition de divers hydrates de carbone soit à l'eau peptonée, soit à l'eau physiologique aminée.* Dans quelques essais, nous avons comparé les effets de l'addition de la glucose ou d'autres hydrates de carbone du groupe des sucres soit à l'eau peptonée, soit à l'eau physiologique aminée. Le tableau VII reproduit une telle expérience.

Il manque malheureusement, dans l'expérience rapportée dans le tableau VII, les tubes témoins formés d'eau peptonée ou d'eau physiologique aminée. Mais, d'après les resultats relatés dans les tableaux II, V et VI et d'après d'autres expériences, nous savons que le développement des colibacilles est, en général, plus considérable et plus précoce en eau peptonée qu'en eau

physiologique aminée.   Par contre, la réaction de l'indol est plus intense et apparaît plus tôt dans les cultures de colibacilles en eau physiologique aminée que dans celles en eau peptonée.   L'eau physiologique aminée ne constitue pas, en outre, un très bon milieu de culture pour les bacilles d'Eberth, qui n'y poussent pas, ou qui n'y poussent guère.   La croissance des bacilles typhiques s'effectue au contraire très bien en eau peptonée, quoique beaucoup plus lentement et de façon bien moins notable que celle des colibacilles dans ce milieu.

En eau peptonée, la croissance des colibacilles a été très intense en présence de rhamnose, de glucose, de galactose, de lactose ou de maltose.   Il en a été de même en présence de saccharose, mais le développement des bacilles s'est effectué un peu moins vite dans ce milieu que dans ceux auxquels nous venons de faire allusion.   Au contraire, le xylose a empêché les colibacilles de pousser en eau peptonée.

On n'a observé la réaction de l'indol que dans la culture de colibacilles en eau peptonée additionnée de saccharose, examinée au bout de 96 heures.   On n'est pas parvenu a découvrir cette réaction dans ce milieu au bout de 24 heures.   Dans les cultures de colibacilles en eau peptonée additionnée de rhamnose, de glucose, de galactose, de lactose ou de maltose, la réaction de l'indol a toujours fait défaut.   Les hydrates de carbone ont donc exercé une action inhibitoire marquée sur cette réaction.

Les bacilles d'Eberth ont poussé très vite et très fort en eau peptonée saccharosée. ' Leur croissance s'est aussi fort bien effectuée, quoique de façon bien moins intense, en eau peptonée additionnée de galactose ou de lactose.   Elle n'a eu lieu que tard en eau peptonée additionnée de rhamnose.   On a obtenu des résultats douteux en eau peptonée glucosée.   Les bacilles typhiques n'ont pas poussé dans l'eau peptonée additionnée de xylose ou de maltose.

Passons aux résultats obtenus en eau physiologique aminée. Sans atteindre le degré si notable de développement observé en eau peptonée additionnée de glucose, de lactose ou de maltose, les colibacilles ont poussé de façon très intense et très précoce en eau physiologique aminée additionnée de l'un ou l'autre de ces

TABLEAU VII

COMPOSITION DES MILIEUX	ÉCHANTILLON EMPLOYÉ	COLIBACILLES						BACILLES TYPHIQUES						REMARQUES
		Première culture					Deuxième culture (Réensemencement sur agar après 24 heures): Degré de croissance après 24 heures	Première culture					Deuxième culture (Réensemencement sur agar après 24 heures): Degré de croissance après 24 heures	
		Degré de croissance après			Réaction de l'indol après			Degré de croissance après			Réaction de l'indol après			
		24 heures	52 heures	96 heures	24 heures	96 heures		24 heures	52 heures	96 heures	24 heures	96 heures		
Eau peptonée + 1% de rhamnose.	premier	+++	+++	+++	—	—	+	—?	+	+	—	—	—	
	deuxième	+++	+++	+++	—	—		+?	—	—	—	—		
de xylose....	premier	+?	—	—	—	—	—	—	—	—	—	—	—	
	deuxième	—?	—	—	—	—		—	—	—	—	—		
de glucose...	premier	+++	+++	+++	—	—	+	+?	+?	+?	—	—	—	
	deuxième	+++	+++	+++	—	—		+?			—	—		
de galactose...	premier	+++	+++	+++	—	—	+	++	+	+	—	—	+	
	deuxième	+++	+++	+++	—	—		++			—	—		
de saccharose	premier	+++	+++	+++	—	+	+	++	++	++	—	—	+	
	deuxième	+++	+++	+++	—			++			—			
de lactose...	premier	+++	+++	+++	—	—	+	++	+	+	—	—	+	
	deuxième	+++	+++	+++	—	—		—			—	—		
de maltose...	premier	+++	+++	+++	—	—	+	—	—	—	—	—	—	
	deuxième	+++	+++	+++	—	—		—			—	—		

(Tableau — orienté verticalement sur la page)

En-tête de colonnes (répété trois fois) : **« Les cultures de colibacilles présentent, après 96 heures, une couleur rosée. »**

Libellé général des lignes : **Eau physiologique aminée + 1 %**

Sucre		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
d'arabinose	premier	−	−	−	−	−	−	+	++	+	++	++	+
	deuxième	−	−	−	−	−	−	+	++	+	++	++	++
de xylose	premier	−	−	−	−	−	−	−	−	−	−	−	−?
	deuxième	−	−	−	−	−	−	−	−	−	−	−	−?
de glucose	premier	+	−	−	++	++	+?	+	+	++	++	++	++
	deuxième	+	−	−	++	++	+?	+	+	++	++	++	++
de lévulose	premier	−	−	−	−	−	+	−	−	−	+?	+?	+?
	deuxième	−	−	−	−	−	−	−	−	−	+?	+?	+?
de galactose	premier	−	−	−	−	−	−	−	−	−	+?	+?	+?
	deuxième	−	−	−	−	−	−?	−	−	−	+?	+?	+?
de lactose	premier	−	−	−	+	+?	−	+	++	++	++	++	++
	deuxième	−	−	−	+	+?	−	+	++	++	++	++	++
de maltose	premier	−	−	−	−	−	−	+	−	−	++	++	++
	deuxième	−		−	−	−	−	+	traces	−	++	++	++
de mannose	premier	−	−	−	−	−	−	+	traces	−	+++	++	++
	deuxième	−	−	−	−	−	−	+	traces	−	+++	++	++

hydrates de carbone. La croissance des colibacilles a été à peu près du même degré en présence d'arabinose. Elle a atteint, au bout de 96 heures, un degré plus considérable en eau physiologique aminée additionnée de mannose qu'en présence des autres hydrates de carbone dont nous venons de parler. L'addition de xylose, de galactose ou de levulose à l'eau physiologique aminée a empêché les colibacilles de pousser.

On a noté une réaction de l'indol très intense en eau physiologique aminée additionnée d'arabinose. Elle a été un peu moins marquée, mais plus précoce, en présence de lactose. On l'a encore constatée de façon nette en présence de glucose. Elle a été tardive et très faible en présence de mannose. Elle a fait défaut dans les cultures de colibacilles en eau physiologique aminée additionnée de maltose.

L'action inhibitoire des sucres vis-à-vis de la réaction de l'indol est, par conséquent, beaucoup moins forte dans les cultures de colibacilles en eau physiologique aminée que dans celle en eau peptonée. Ceci tient peut-être à la présence de glycyltryptophane dans le premier de ces milieux et de tryptophane dans le deuxième, soit à une plus forte teneur en tryptophane de l'eau physiologique aminée, soit à l'absence dans ce dernier milieu d'autres composés à effets inhibitoires contenus dans l'eau peptonée.

Les bacilles d'Eberth n'ont poussé que faiblement et tardivement en eau physiologique aminée additionnée de lactose. Ils ont poussé de façon plus précoce en eau physiologique aminée additionnée de 1% de glucose ou de lévulose et le degré de croissance est même devenu relativement notable entre la 24ème et la 52ème heure dans un tube d'eau physiologique aminée glucosée. La croissance de ces microorganismes ne s'est pourtant pas toujours effectuée en présence de ces sucres, d'après les données expérimentales relatées dans le tableau VII. Il y a, en outre, lieu de rappeler que nous n'avons jamais observé le moindre développement de bacilles typhiques dans l'eau physiologique aminée renfermant seulement 0.2% de glucose (tableau V). Les bacilles d'Eberth n'ont pas poussé en eau physiologique aminée additionnée de 1% d'arabinose, de xylose, de galactose, de maltose ou de mannose.

On devait se demander si les effets inhibitoires de plusieurs hydrates de carbone sur la réaction de l'indol ne provenaient pas du fait qu'au fur et à mesure de son développement aux dépens du tryptophane, l'indol se combinerait immédiatement à de l'acide glycuronique provenant d'une action éventuelle des colibacilles sur les sucres ajoutés à l'eau peptonée ou à l'eau physiologique aminée. Nous avons en vain recherché au moyen des diverses réactions des acides glycuroniques (avec l'orcine, avec la phloroglucine, avec la chlorure de calcium et l'acide chlorhydrique) à demontrer la présence d'acide indoxylglycuronique. Il nous est donc impossible d'expliquer pour le moment pourquoi l'on ne parvient pas à mettre en évidence la réaction de l'indol dans les cultures de colibacilles soit en eau peptonée additionnée de rhamnose, de glucose, de galactose, de lactose ou de maltose, soit en eau physiologique aminée additionnée de maltose.

Rappelons que, d'après Haenen, la présence de glucose ou de lactose dans les cultures de colibacilles en bouillon peptoné n'empêcherait pas de découvrir l'indol au moyen de l'aldéhyde paradiméthylaminobenzoïque, contrairement à ce qui serait le cas dans le réaction classique de l'indol au moyen d'une nitrite et d'un acide (Bleisch 1893, Gorini 1893, Smith 1893, Seelig 1897).

B. *Expériences effectuées avec d'autres microorganismes que les colibacilles et les bacilles d'Eberth*

Il nous reste à parler de quelques recherches faites avec les bacilles paratyphiques A et B, les vibrions du choléra, les bacilles pyocyaniques, les staphylocoques pyogènes dorés et les bacilles de l'entérite de Gärtner. Dans l'expérience relatée dans le tableau VIII l'on a comparé la croissance de ces microorganismes et aussi celle des colibacilles et des bacilles typhiques pris comme témoin, d'une part en bouillon additionné soit de peptone soit de glycyltryptophane, d'autre part en eau physiologique peptonée soit telle quelle, soit additionnée de glycyltryptophane. On a aussi recherché la réaction de l'indol dans les divers tubes ainsi preparés.

TABLEAU VIII

ESPÈCE DE MICRO-ORGANISMES UTILISÉ LORS DE L'ENSEMENCEMENT	ÉCHANTILLON EMPLOYÉ	BOUILLON								EAU PHYSIOLOGIQUE PEPTONÉE A 1%							
		Peptoné				Sans peptone, mais contenant 0.3% de glycyltryptophane				Telle quelle				Additionnée de 0.3% de glycyltryptophane			
		Degré de croissance après		Réaction de l'indol après		Degré de croissance après		Réaction de l'indol après		Degré de croissance après		Réaction de l'indol après		Degré de croissance après		Réaction de l'indol après	
		36 heures	96 heures	36 heures	96 heures	36 heures	96 heures	36 heures	96 heures	36 heures	96 heures	36 heures	96 heures	36 heures	96 heures	36 heures	96 heures
Colibacilles	premier	+++	++++	—	+	++	+++	+++	+	++	+	+	++	+++	++	++	+++
	deuxième	+++	++++	—	—	++	+++	+++	—	++	+	—	—	+++	++	++	+++
Bacilles typhiques	premier	+++	+++	—	—	++	+	—	—	+?	+	—	—	+++	++	—	—
	deuxième	+++	+++	—	—	++		—	—	+?		—	—	+++	++	—	—
Bacilles paratyphiques A	premier	+++	+++	—	—	+++	++	—	—	+++	+	—	—	+++	++	—	—
	deuxième	+++	+++	—	—	+++		—	—	+++		—	—	+++		—	—
Bacilles paratyphiques B	premier	++++	+++	—	—	+++	+	—	—	+?	+?	—	—	+++	+	—	—
	deuxième	+++	+++	—	—	++		—	—	+?		—	—	+++		—	—
Vibrions du choléra	premier	—	—	—	—	—?	++	+	+	—?	—	—	—	+++	+++	+++	++
	deuxième	—	—	—	—	—?		—	—	—?		—	—	+++		+++	++
Bacilles pyocyaniques	premier	+++++	++	—	—	—	++	—	—	++	+	—	—	+++	++	—	—
	deuxième	+++++		—	—	++		—	—	++		—	—	+++		—	—
Staphylocoques pyogènes dorés	premier	+++	+++	—	—	++	++	—	—	++	+	—	—	++	+	—	—
	deuxième	+++		—	—	++		—	—	++		—	—	++		—	—
Bacilles de l'entérite de Gärtner	premier	+++++	++++	—	—	++	+++	—	—	++	+	—	—	+++	++	—	—
	deuxième	+++++		—	—	++		—	—	++		—	—	+++		—	—

Les vibrions du choléra n'ont poussé que dans l'eau physiologique peptonée additionnée de glycyltryptophane.

Tous les autres microorganismes ont manifesté le maximum de croissance en bouilllon peptoné et le minimum en eau peptonée. Les bacilles d'Eberth, les bacilles paratyphiques B et les staphylocoques pyogènes dorés n'ont pas, ou n'ont guère poussé dans ce dernier milieu.

Par ordre décroissant d'intensité de développement, on doit ranger, pour les colibacilles, les staphylocoques pyogènes dorés et les bacilles de l'entérite de Gärtner, de la manière suivante, les quatre milieux étudiés: bouillon peptoné, bouillon additionné de glycyltryptophane, eau peptonée additionnée de glycyltryptophane, eau peptonée. Il en est de même pour les bacilles paratyphiques A, sauf que la croissance de ces microorganismes a été la même en bouillon additionné de glycyltryptophane et en eau peptonée additionnée de ce peptide. Les bacilles pyocyaniques ont manifesté un plus grand degré de développement au bout de 96 heures en bouillon additionné de glycyltryptophane qu'en eau peptonée additionnée ou non de ce peptide, alors qu'au bout de 36 heures l'on constatait déjà une croissance marquée de cette espèce de microorganismes dans les deux derniers milieux, mais rien en bouillon additionné de glycyltryptophane. Les bacilles d'Eberth et les bacilles paratyphiques B se sont developpés davantage en eau peptonée additionnée de glycyltryptophane qu'en bouillon additionné de ce peptide.

Si nous comparons l'intensité de développement des diverses espèces microbiennes dans le milieu le plus favorable à leur croissance, c'est-à-dire le bouillon peptoné, nous les rangerons dans l'ordre décroissant suivant: (1) bacilles pyocyaniques et bacilles de l'entérite de Gärtner; (2) colibacilles et bacilles paratyphiques B; (3) bacilles d'Eberth, bacilles paratyphiques A et staphylocoques pyogènes dorés. Il s'agit d'une simple constatation, car rien ne prouve que cet ordre soit toujours le même dans des conditions expérimentales identiques.

On n'a observé la réaction de l'indol que dans les cultures de colibacilles et de vibrions du choléra. Pour les colibacilles, la réaction a présenté le maximum d'intensité et de précocité dans

le bouillon additionné de glycyltryptophane, l'eau peptonée, le bouillon peptoné.   Il semble bien que la peptone de Witte et le bouillon renferment des composés qui entravent soit la formation de l'indol aux dépens du tryptophane, soit la mise en évidence de l'indol au moyen de l'aldéhyde paradiméthylaminobenzoïque selon la méthode préconisée par Haenen.

IV. *Considérations générales*

Les résultats expérimentaux relatés ci-dessus provoquent assurément bien des remarques.   Mais comme ces recherches demandent à être étendues et complétées à bien des points de vue, nous nous bornons à insister sur quelques faits qui paraissent en ressortir avec une netteté suffisante.

Tout d'abord, les colibacilles sont bien moins exigeants pour leur culture que les bacilles d'Eberth.   C'est, du reste, une chose bien connue.

Les bacilles typhiques ne se sont pas developpés dans l'eau physiologique ayant comme seule source d'azote soit un acide aminé, soit un peptide, soit une protéose.   On ne constate de développement appréciable de ces microorganismes en eau physiologique qu'en présence d'une protéine déjà complexe, la gliadine (tableau II).   Les bacilles d'Eberth parviennent, il est vrai, à pousser faiblement dans l'eau physiologique contenant de la xanthine ou de la taurine (tableau III).

Les colibacilles se développent fort bien dans l'eau physiologique renfermant un seul corps azoté approprié.   Un acide monoaminé suffit parfaitement dans ce but.

Contrairement à ce qu'Armand-Delille, A. Mayer, G. Schaeffer et E. P. Terroine ont observé pour les bacilles tuberculeux, les colibacilles parviennent à se développer dans un milieu ne renfermant comme source azotée qu'un acide aminé à noyau aromatique, la phénylalanine.   Ce développement est toutefois bien moins accentué que lorsqu'on part d'un acide monoaminé de la série grasse.

D'après les résultats consignés dans les tableaux I et II, la leucine paraît être, parmi les acides monoaminés, la meilleure

source azotée de développement pour les colibacilles, alors que les expériences des auteurs cités ci-dessus tendent à démontrer que le glycocolle joue ce rôle pour les bacilles tuberculeux.

En partant du bouillon comme base du milieu de culture, on observe qu'une protéine (la gliadine), une protéose (l'hétéroalbumose), un peptide (le glycyltryptophane) fournissent une culture aussi abondante de colibacilles que le bouillon additionné de leucine. Aucun de ces milieux n'amène de développement comparable à celui de ces microorganismes en bouillon peptoné a 1%. Armand-Delille, A Mayer, G. Schaeffer et E. P. Terroine ont au contraire constaté qu'une solution a 0.2% de glycocolle dans le bouillon de boeuf donne une culture de bacilles tuberculeux aussi abondante que le bouillon peptoné a 1%.

Si l'on prend l'eau physiologique comme base du milieu de culture, la leucine amène un développement tout aussi abondant des colibacilles que l'eau peptonée et ce développement s'effectue même plus rapidement que dans ce dernier milieu. Si l'on ajoute à l'eau physiologique un autre acide aminée, un peptide, une protéose, une protéine permettant la croissance des colibacilles, on n'observe pas par contre de développement aussi intense de ces microorganismes qu'en eau peptonée.

Tandis qu'une seule source d'azote suffit à un développement très net du colibacille, · des expériences d'Armand-Delille, A. Mayer, G. Schaeffer et E. P. Terroine ont établi que deux sources d'azote distinctes sont indispensables pour la croissance régulière du bacille de Koch, à savoir, un acide monoaminé, le glycocolle, et un acide diaminé, l'arginine.

On obtient un développement plus abondant des colibacilles si l'on multiplie les sources d'azote, c'est-à-dire si l'on emploie par exemple de l'eau physiologique renfermant un peptide (le glycyltryptophane) et soit un acide diaminé (l'histidine), soit une ou plusieurs substances extractives (créatine et sarcosine).

Il se pourrait fort bien que la peptone de Witte intervienne dans le développement des colibacilles par l'apport de certains éléments azotés et surtout par sa teneur en leucine.

Nous avons effectué plusieurs milieux artificiels dans lesquels les colibacilles poussent rapidement et en grande quantité. Mais

nous n'avons malheureusement pas comparé ces milieux (tableau VI) à l'eau peptonée ou au bouillon peptoné.   Nous ne pouvons donc affirmer qu'ils soient préférables à ces deux derniers milieux au point de vue de la croissance des colibacilles.

Par contre, en comparant les données des tableaux I, II, V, VI, VII et VIII, on constate que de l'eau renfermant 0.5% de chlorure de sodium, 0.2% de glycyltryptophane, 0.2% de glucose, 0.2% de sarcosine, 0.2% de créatine, 0.2% de bétaïne et 0.2% de xanthine paraît tout particulièrement appropriée à la recherche de l'indol dans les cultures de colibacilles.

Peut-être vaut-il même mieux préférer, pour la différenciation des colibacilles d'avec les bacilles d'Eberth au moyen de la réaction de l'indol, à ce milieu, qu'on peut déjà qualifier de relativement complexe, de l'eau contenant en solution 0.5% de chlorure de sodium, 0.2% de glycyltryptophane et 0.2% d'histidine.   En effet, si les résultats relatés dans le tableau VI se vérifient, un tel milieu donnerait la réaction de l'indol de façon très précoce. D'autre part, les colibacilles y poussent certes moins bien que dans le milieu précédent, mais apparaissent néanmoins bien avant les bacilles d'Eberth.

Il appartiendra à de nouvelles recherches de déterminer le milieu à préférer dans le but dont nous venons de parler.   On ne saurait être trop prudent à cet égard.   Il convient d'expérimenter avec de multiples souches de colibacilles et de bacilles d'Eberth et d'examiner, toutes les heures après l'ensemencement, la réaction de l'indol dans ces milieux en les comparant à l'eau peptonée et au bouillon peptoné, tels quels ou additionnés de glycyltryptophane.   Rien ne prouve, du reste, qu'il n'y ait pas intérêt à changer la proportion de l'un ou l'autre des parties constituantes des deux milieux dont nous venons de nous occuper, ou même de remplacer l'un ou l'autre des composés azotés renfermés dans ces milieux par un composé plus approprié.

En effet, nous n'avons, malheureusement pas étudié les effets exercés par l'addition à l'eau physiologique aminée de leucine et d'autres acides monoaminés sur la croissance des colibacilles et sur la réaction de l'indol dans leurs cultures.   Or, la leucine a, en eau physiologique, une action très favorable sur le développe-

ment des microorganismes sans amener de croissance des bacilles d'Eberth. Tel est aussi le cas de l'histidine. Dès lors, on doit se demander si l'addition simultanée de leucine et d'histidine à l'eau physiologique aminée ne réaliserait pas un milieu en quelque sorte idéal pour la différenciation des colibacilles d'avec les bacilles typhiques au moyen de la réaction de l'indol. Nous ne manquerons pas de nous préoccuper de cette question dès que les circonstances nous le permettront.

Nos expériences mettent une fois de plus en lumière que la formation d'indol dans les cultures de colibacilles dépend de la teneur du milieu en tryptophane. La présence de glycyltryptophane dans le milieu de culture paraît particulièrement appropriée à une formation intensive d'indol tant par les colibacilles que par les vibrions du choléra.

Un autre point que nous croyons devoir souligner, c'est la forte action inhibitoire de l'inosite sur le développement tant des colibacilles que des bacilles d'Eberth. Il suffit, pour s'en rendre compte, de comparer les résultats obtenus dans le tableau VI par l'addition d'inosite soit au milieu formé d'eau physiologique, de glycyltryptophane, de glucose et d'histidine, soit au milieu composé d'eau physiologique, de glycyltryptophane, de glucose, de sarcosine, d'alloxane et de taurine. Il serait certes fort intéressant d'entreprendre des recherches in vivo à propos de cette action inhibitoire de l'inosite.

Signalons encore que la réaction de l'indol au moyen de l'aldéhyde paradiméthylaminobenzoïque est entravée par diverses substances et surtout, dans certaines conditions, par plusieurs espéces d'hydrates de carbone.

RÉSUMÉ

1. Les bacilles typhiques ont de plus grandes exigences pour leur nourriture azotée que les colibacilles.

2. Les colibacilles parviennent à se développer dans l'eau physiologique ne renfermant qu'un acide monoaminé comme source d'azote. La leucine permet des cultures extrêmement riches de ces microorganismes.

3. Les bacilles typhiques ne se développent pas dans l'eau physiologique renfermant un acide aminé proprement dit, un peptide ou une protéose, mais bien en présence de certaines protéines ou de certains composés azotés tels que la sarcosine, la taurine et la xanthine. La gliadine constitue un substrat azoté particulièrement favorable à la culture de ces microorganismes.

4. L'inosite entrave beaucoup le développement tant des colibacilles que des bacilles typhiques dans divers milieux de culture.

5. La formation de l'indol dans les cultures de colibacilles dépend de leur richesse en tryptophane. La présence de glycyltryptophane dans les milieux de culture est très utile pour la recherche de la réaction de l'indol.

6. Certains hydrates de carbone entravent la réaction de l'indol dans les cultures de colibacilles.

7. On peut substituer à l'eau peptonée des milieux chimiquement définis très favorables à la recherche de la réaction de l'indol dans les cultures de colibacilles. Les deux meilleurs milieux experimentés ont été les solutions aqueuses suivantes: (1) 0.5% de chlorure de sodium, 0.2% de glycyltryptophane, 0.2% d'histidine; (2) 0.5% de chlorure de sodium, 0.2% de glycyltryptophane, 0.2% de glucose, 0.2% de sarcosine, 0.2% de créatine, 0.1% bétaïne et o.2% de xanthine.

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SOME REGULATING FACTORS IN BACTERIAL METABOLISM

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Kendall and his co-workers, in a series of investigations, have developed the idea that in the metabolism of bacteria sugar has a sparing effect on the digestion of protein and that, consequently, the continuous feeding of sugar to animals would induce a change in the intestinal flora from a proteolytic to an acid forming type. These results on sugar feeding were confirmed recently by Rettger working on white mice and chickens and by Torrey on human typhoid patients.

In his investigations Kendall used a constant proportion of sugar to peptone (1 per cent of each) in standard nutrient broth. Observations, made in the course of a study on the proteolytic enzymes of the *B. proteus*, in media in which the concentration of sugar was varied, led me to think, however, that there were other important factors involved in bacterial metabolism, the actual effects of which were not known.[1] It became quite evident from these and other observations that the relative concentrations of sugar and peptone were significant; that different organisms of closely related groups were capable of utilizing varying amounts of sugar, and that the amount of sugar that a particular form could completely ferment varied within limits with the concentration of the other ingredients, especially peptone and phosphate salts. The following are the results of a series of preliminary experiments to test more definitely the validity of these observations:

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[1] Kendall and Walker in an independent investigation of the same problem obtained identical results.

A. Effect of different concentrations of peptone on the nitrogen utilization of certain intestinal bacteria. The best measure of the degree of bacterial metabolism in nitrogenous media is the amount of ammonia liberated. This criterion was therefore used. The technique was, briefly, as follows:

Simple solutions of peptone in distilled water containing varying amounts of peptone and 0.5 per cent glucose were used. Two series were run; one with Witte's and the other with an American peptone. The different lots of media from each peptone were made at the same time, flasked, and autoclaved under the same conditions. Inoculations were made from young cultures and the flasks incubated at 30°C. for seven days. One lot of Witte peptone was incubated for five days, but this period was found insufficient.

The ammonia determinations were made according to the Folin micro method. First, 5 cc. of the culture were used but later 10 cc. were found more satisfactory, due to the small amounts of ammonia present. The period of aeration was twenty minutes. The ammonia was caught in 10 cc. $\frac{N}{50}$ sulphuric acid and the excess acid titrated with $\frac{N}{50}$ Na(OH).

The effect of different concentrations of peptone is evident from the results of this experiment. It is, of course, possible that the greater ammonia production with the higher concentration of peptone is due merely to the favorable action of the increased amount of buffer in keeping down the hydrogen ion concentration. That the concentration of the peptone is in itself an important factor, is however indicated by the different results obtained with different types of bacteria. *B. typhi*, for instance, produced no ammonia in the 0.5 per cent peptone in one medium and a negative quantity in the other, while in the higher concentrations small amounts were obtained, though less than in the sugar-free control. *B. coli* and *B. cloacae*, etc., give increasing amounts of ammonia with the increasing concentration of peptone; but in all cases the amount still remains lower than in the sugar-free control.

The results, on the whole, indicate that the concentration of peptone is an appreciable factor when the concentration of glucose is moderately low. Attention may also be called in passing

to the different results with the two peptones.   This is, no doubt,
due to an essential difference in the composition of the two prod-
ucts.   Since Rettger has shown that certain bacteria do not

TABLE 1

*Amount of ammonia produced in solutions containing different amounts of Witte's
peptone and 0.5 per cent sugar*

ORGANISM	AMOUNT OF NH₃ IN CGM. IN 100 CC. OF MEDIA*							
	0.5% peptone 0.5% glucose		1.0% peptone 0.5% glucose		1.5% peptone 0.5% glucose		1.5% peptone No glucose	
	5 days	7 days	5 days	7 days	5 days	7 days	5 days	7 days
B. coli....................	−0.17	0.17	0.0	0.34		0.68	0.34	0.34
B. cloacae................	0.17	0.51	0.17	0.68		0.85	1.02	0.68
B. paracoli...............	−0.51	0.00	0.00	0.17	0.34	0.17	0.85	0.51
B. capsulatus.............	0.17	0.17		0.51	0.17	0.34	0.85	0.68
B. typhi..................	−0.68	0.00	−0.17	0.00	0.00	0.00	0.17	0.34
Uninoculated control......	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

* The data presented here were obtained from two distinct sets of tests run
on different occasions.   In the first a five-day and in the second a seven-day
period of incubation was used.   The effects of the different amounts of peptone
on the ammonia yield are brought out in both series.

TABLE II

*Amount of ammonia produced in solutions containing different amounts of American
peptone and 0.5 per cent glucose*

ORGANISM	AMOUNT OF NH₃ IN CGM. IN 100 CC. OF MEDIA			
	0.5% peptone 0.5% glucose	1% peptone 0.5% glucose	1½% peptone 0.5% glucose	Control, 1% Peptone, no sugar
	7 days	7 days	7 days	7 days
B. coli................................	0.17	0.17	0.34	2.04
B. cloacae............................	0.34	0.51	0.68	2.38
B. paracoli............................	0.00	0.00	0.17	1.19
B. typhi..............................	0.00	0.17	0.17	0.51
B. capsulatus.........................	0.17	0.17	0.68	1.70
Uninoculated control..................	0.00	0.00	0.00	0.00

digest proteoses and peptones, it seems that the American
product is very likely richer in amino-acids and hence gives a
higher yield of ammonia in all instances.

   *B. Effect of different concentrations of peptone and glucose and
phosphate on the metabolism of intestinal bacteria.*   Another index

of the metabolism of bacteria is the change they produce in the hydrogen ion concentration of the medium as a result of their activities. The acid titration or the ammonia determination indicates only the action on either the carbohydrates or the peptone present in the medium. The hydrogen ion concentration, on the other hand, gives the resultant of the action on both the carbohydrate and nitrogenous components. By determining the hydrogen ion concentration on different days, one may even trace the progressive increase with the active utilization of the sugar and subsequent decrease, if any occurs, during the active utilization of the constituents of the peptone. This test, furthermore, serves as a simple index of (a) the amount of sugar a particular organism may utilize without producing sufficient acid to inhibit further growth and (b) the rate at which different organisms can utilize a particular sugar. Experiments are under way which indicate that distinct and constant differences exist in both properties among closely related types.

The method with some slight modifications is that used by Clark and based on Sorensen's colorimetric method of determining the hydrogen ion concentration. Standard solutions of primary and secondary phosphate and of sodium acetate-acetic-acid and the indicators described by Clark and Lubs were used.

Media were made as above containing varying amounts of peptone, glucose and phosphate. A series of typical cultures was inoculated into these media, incubated at 30° C. and tests made at regular intervals. In order to eliminate the color, due to the breaking down of the glucose during sterilization it was found necessary to sterilize the peptone-phosphate solution and the sugar solution separately, and then add the sugar to the peptone by means of sterile pipettes. This somewhat increased the difficulty in making the medium but assured a water-clear solution, which greatly facilitated the color readings. For testing the hydrogen ion concentration 1 cc. of the culture was mixed with 5 cc. of freshly distilled water[2] and three to four drops of the indicator added.

[2] Clark has suggested a dilution of 1 : 10 but a few preliminary tests showed that it was more desirable to use a 1 : 5 dilution as excessive dilution changes the hydrogen ion concentration.

(1) *Effects of different amounts of peptone with and without the addition of phosphate.* The object of this experiment was to determine the effects of different concentrations of peptone on the carbohydrate and nitrogen utilization by bacteria and the influence exerted by the addition of an acid regulator such as primary sodium phosphate ($Na_2H PO_4$). The media were made as outlined above and tests for the hydrogen ion concentration run on the second and fourth days. With a little care 1 cc. of the culture can be withdrawn by means of a sterile pipette without contaminating it. By this simple method the progressive changes in the same culture tube could be followed for a week or more by daily withdrawals of 1 cc. samples.

The results of these tests are summarized in table III. They indicate quite clearly that the concentration of peptone plays a rather significant rôle in accelerating glucose fermentation, as well as in favoring its complete utilization. The former effect is seen in table III, section A, in the differences in the P_H^+ values in the *B. cloacae* cultures on the second day and in the *B. aerogenes* cultures on the fourth day. A similar effect is noted in table III, section B, in the case of *B. coli*. The favorable effect on complete utilization of the carbohydrate is brought out especially in the case of *B. aerogenes* in section A and of *B. coli* in section B of table III.

The effect of the regulating action of the acid-phosphate is evident on comparing sections A and B. By keeping down the hydrogen ion concentration of the medium some of the organisms are enabled to use up all the carbohydrate (0.5 per cent) without producing sufficient concentration of acid to inhibit their growth. Once this is accomplished, the active utilization of the nitrogenous constituents commences and we obtain a progessively decreasing hydrogen ion concentration (or an increasing P_H^+ value). That the regulating effect of the phosphate is not the only factor, however, is evident from the results obtained with *B. coli* as shown in table III.

It is interesting to note that in the absence of phosphate the cultures of *B. cloacae* and *B. aerogenes* reach a hydrogen ion concentration practically as high as that of *B. coli*, which does

not occur in the same medium when 0.5 per cent phosphate is present. Another interesting point is that the final hydrogen ion concentration is higher when no phosphate is present; the primary phosphate obviously tends to keep down the concentration of the hydrogen ions.

TABLE III

The effects of different amounts of peptone, with and without phosphate, on the utilization of the carbohydrate and peptone, as indicated by the hydrogen ion concentration

		COMPOSITION OF THE MEDIA											
		A						B					
Phosphate		0.0%		0.0%		0.0%		0.5%		0.5%		0.5%	
Glucose		0.5%		0.5%		0.5%		0.5%		0.5%		0.5%	
Peptone		0.5%		1.0%		1.5%		0.5%		1.0%		1.5%	
		P_H^+		P_H^+		P_H^+		P_H^+		P_H^+		P_H^+	
Days		2	4	2	4	2	4	2	4	2	4	2	4
Cultures	B. coli*		4.8		4.6		4.6	4.8	5.1	5.4	6.6	6.0	6.6
	B. paracoli	4.6	4.8	4.6	4.8	4.8	4.6	5.0	5.2	5.2	5.2	5.3	5.0
	B. cloacae	5.2	5.6	5.6	6.6	6.4	6.8	6.5	6.8	6.6	7.0	6.8	7.2
	B. aerogenes	4.6	4.6	4.8	5.0	4.8	5.6	6.6	6.6	6.4	6.8		
	B. typhi	4.8	4.6	4.6	4.7	4.6	4.8	5.0	5.1	4.8	5.0		

* This strain of *B. coli* gave these results constantly. Observations in connection with other experiments indicate that not all strains behave in this manner. It is quite likely that there are two types of *B. coli* (corresponding to the *B. communior* and *B. communis* respectively), which may be differentiated in this way.

(2) *Effect of different concentrations of phosphate on the metabolism of bacteria.* The results of the experiments recorded above indicated that primary phosphate, acting apparently as an acid regulator, played an important part in controlling the nutritive processes of bacteria. In order to get more light on the nature of this regulatory mechanism and its influence on the carbohydrate and peptone utilization the following tests were performed:

Media were made containing a constant and optimum amount of peptone (1 per cent), comparatively low concentrations of

glucose (0.3 per cent and 0.4 per cent, respectively), and varying amounts of phosphate salt, (0.2 per cent, 0.3 per cent and 0.4 per cent, respectively). As nearly as possible the methods of preparation of media, inoculation, incubation, etc., were kept uniform. Tests were made on the first, third, and fourth days, respectively, with the results given in table IV.

TABLE IV

Effect of different concentrations of acid phosphate on the utilization of glucose by bacteria

	A			B		
Peptone................... Glucose................... Phosphate.................	1.0% 0.3% 0.2%	1.0% 0.3% 0.3%	1.0% 0.3% 0.4%	1.0% 0.4% 0.2%	1.0% 0.4% 0.3%	1.0% 0.4% 0.4%
No. of culture	P_H^+ value	P_H^+ value	P_H^+ value	P_H^+ value	P_H^+ value	P_H^+ value
11.................	4.9	4.9	5.0	4.9	5.0	5.1
16.................	*4.7*	4.8	*6.2*	4.7	4.8	5.5
17.................	*5.0*	5.0	*6.5*	4.6	4.6	5.0
18.................	*6.0*	6.4	*7.3*	*4.8*	4.6	*7.4*
19.................	*5.0*	*6.2*	*6.8*	*4.8*	4.7	*7.2*
22.................	*4.9*	5.2	*6.5*	4.8	4.8	5.0
24.................	6.6	6.6	7.4	*6.2+*	*6.2+*	7.4
40.................	6.6	6.6	*6.8*	*6.2+*	*6.2+*	6.8
44.................	4.9	5.0	5.4	4.8	4.9	4.9
135.................	*5.6*	5.8	*6.6*	*4.9*	4.7	*6.5*
196.................	4.8	5.4	5.6	5.4	5.5	5.2
239.................	*5.0*	5.0	*6.4*	4.8	4.8	5.0

To avoid the confusion that might result from unnecessary detail only the four-day tests are tabulated. The effect of the phosphate is strikingly shown in both the A and B section of the Table. The significant results are italicized. The low figures represent high hydrogen ion concentrations—or the acid phase—while the high figures represent low hydrogen ion concentrations or the alkaline phase. The progressive change produced in carbohydrate media by members of the colon-typhoid group, excepting certain types, is from the alkaline to acid and back. So long as there remains unutilized carbohydrate the acid phase persists. With the complete consumption of the

carbohydrate the organism actively attacks the nitrogenous components of the medium, neutralizing the acid and gradually returning to the alkaline phase.   The phosphate evidently takes care of the free acid, thus keeping the ionic concentration below the lethal point and enabling the organism to proceed in its activity.   As a result of this regulative power the amount of sugar which a particular species can completely utilize varies within limits with the relative amount of primary phosphate salts present in the medium.   Thus, in the presence of only 0.2 per cent phosphate, 0.3 per cent sugar is completely digested (as indicated by the low hydrogen ion concentration) by only four organisms (18, 24, 40 and 135) while only two (24 and 40) can use up 0.4 per cent glucose.   In the presence of 0.3 per cent phosphate on the other hand 0.3 per cent glucose is also broken down by number 19; and when 0.4 per cent phosphate is added all but one are capable of completely destroying 0.3 per cent sugar, while five (18, 19, 24, 40 and 135) can even use up 0.4 per cent glucose.   On increasing the amount of sugar to 0.5 per cent even in the presence of 0.5 per cent phosphate only 19, 24 and 40 reach the alkaline phase (see table III).

C. *Amount of sugar digested by different species.*   Aside from the effect of varying concentrations of phosphate, the different types manifest specific differences in their power to digest definite quantities of glucose under a given set of conditions.   These differences are not attributable to the toxic action of the high hydrogen ion concentration on the cell, alone.   Why one organism (11, for instance) (see table IV) should not be able to use 0.3 per cent glucose while number 18 will do away with 0.4 per cent with the same amount of phosphate (see table IV), when both reach approximately the same limiting hydrogen ion concentration (4.8 and 4.9, respectively, table IV, section B), is not evident on the surface.   The difference is partly explicable on the basis that number 18 (*B. enteritidis*) carries the fermentation further than number 11 (*B. typhi*), thus removing the acid and its inhibitive effects.   But this would not explain the differences between numbers 17, 18 and 19, for instance, all gas-producing forms.   We must assume either that there are inhibiting factors

of a specific character or else that specific differences exist in the way the glucose molecule is split by these different forms giving rise to different amounts of toxic substances.   The interesting fact remains that, under the same conditions, these closely related forms manifest strikingly different powers of utilization of sugar.   Such a difference exists between *B. aerogenes* and *B. coli*, and Clark, taking advantage of this has devised a simple method for differentiating the two under a given set of conditions.   The difference between *B. aerogenes* and *B. coli* is, perhaps, more fundamental than that between the other members of the group.   Specific differences do exist, however, and experiments are now well under way, which promise to furnish a basis for separating certain of the other more closely related members of this group.

SUMMARY AND CONCLUSIONS

A study of some of the factors involved in the regulation of bacterial metabolism was made.   The effects of different concentrations of peptone on the utilization of glucose and aminoacids, and of different concentrations of phosphate on the fermentation of glucose were studied.   The nature of the metabolic changes was measured by ammonia determinations by the Folin method and by noting the change in the hydrogen ion concentration by the Sorensen-Clark method.   The results were, briefly, as follows:

1. The concentration of peptone is an appreciable factor, controlling the nutrition of bacteria.   With a moderate amount of glucose present the higher the concentration of peptone the greater the amount of ammonia produced.   The amount of ammonia is in no case as great as in the sugar-free control, indicating a distinct sparing effect.

2. Primary phosphate acting as an acid-regulator plays a very important part in the regulating of the carbohydrate utilization by different bacteria of the colon-typhoid group.

3. The different members of the colon-typhoid group manifest specific differences in their power of completely utilizing different amounts of sugar under a given set of conditions. These differences may be utilized for species differentiation.

BOOK REVIEW

Practical Textbook of Infection, Immunity, and Specific Therapy. John A. Kolmer, M.D., Dr. P.H. Octavo of 899 pages with 143 illustrations, 43 in colors. Philadelphia: W. B. Saunders Company. 1915. $6. Half morocco, $7.50.

Kolmer's work has not been in print a year, but has already won an important place among the books useful to practitioner and laboratory worker.

It is well-planned and, throughout, has the stamp of having been written by one who is actively working in the subject about which he is writing. It therefore avoids the fault, so frequently apparent in textbooks, of consisting largely of the warmed-over teaching gleaned from other writers. Kolmer is active in his subject, and has the judgment of the trained worker in selecting his material.

The discussions of theory are not too extensive and indeed in the probably intentional neglect of detailed presentation of evidence on controversial questions, the book may be somewhat disappointing to the specializing bacteriologist and serologist. However, one has the feeling that it is Kolmer's purpose to furnish a very complete manual for the scholarly practitioner without confusing him by too involved a discussion of principle, and this purpose is excellently served by the book as a whole.

The work is divided into five parts treating respectively of general immunologic technique, principles of infection, principles of immunity, applied immunity, prophylaxis and treatment, and a series of experiments excellently compiled for teaching purposes. Especially satisfactory are those parts of the book which deal with clinical applications, for the author seems to possess much judgment in matters in which his own subject has contact with the clinic, an attribute not over-common among laboratory teachers. The style is clear, illustrations as a whole are of more than ornamental value, and explanations are concise.

The book should fulfill an important didactic function in making easily accessible the principles underlying tests and procedures which are used in hospital, clinic, and private practice, and which, unfortunately, are too often ordered by the attending physician without adequate knowledge concerning the principles underlying their performance and interpretation. Perhaps there has never been an era in medicine in which the physician has relied so much upon the aid of diagnostic and therapeutic measures of which he possessed so little direct understanding, and many a learned and affluent practitioner sends for typhoid serum when he means vaccine, and thinks that the Wasser-

mann reaction is a sort of Widal test done with the *Treponema palli-dum.* In correcting this state of affairs, the author will be a valuable missionary, since the entire tone of the work is one which will appeal to the practicing physician and give him such a wealth of interesting knowledge, that he will read it and after all, the fact that it will be read is not the least important quality of a printed book.

HANS ZINSSER.

ABSTRACTS OF AMERICAN BACTERIOLOGICAL LITERATURE

BACTERIOLOGY OF SOILS

Effect of Grinding Soil on the Number of Microörganisms. E. B. FRED. Science, 1916, **44**, 282–283.

The soil was ground in a ball mill for varying periods of time. Very marked reductions were obtained after one hour and an "enormous decrease" followed eight or twenty-four hours grinding. Soil protozoa were also destroyed by grinding.—C. M. H.

Preliminary Investigations in Comparison of Field with Laboratory Experiments in Soil Biology. G. P. KOCH. Soil Science, 1916, **2**, 87–92.

Biological experiments (e.g., in ammonification) can be successfully carried out in the field. As a rule, a greater amount of organic matter seems to be ammonified in the laboratory tests than in the field, and field studies of nitrogen fixation and nitrification are greatly interfered with by rains. The nitrogen content of the soil varies considerably, even over a comparatively small area.—Z. N.

Sources of Error in Soil Bacteriological Analysis. H. C. LINT AND D. A. COLEMAN. Soil Science, 1916, **2**, 157–162.

A comparison is made of the soil shaker with the spatula method of mixing soil for bacteriological analysis, to the great advantage of the former. The experimental error is smaller with the soil shaker. The shaker is easily sterilized; it is also exceptionally well adapted to the incorporation into soil of very small quantities of materials in a finely pulverized condition.—Z. N.

The Effect of Soil Reaction on Ammonification by Certain Soil Fungi. N. KOPELOFF. Soil Science, 1916, **1**, 541–573.

Three soil fungi, *Rhizopus nigricans*, *Zygorhynchus vuilleminii* and *Penicillium* sp. 10, were studied as to their reaction requirements in soil. They possess a comparatively narrow range of reaction tolerance for maximum ammonification which was found to be between the neutral point and an acidity equivalent to 2000 pounds CaO per acre. This was true whether sandy or clay soils were used with either dried blood or cottonseed meal.

Where the soil reaction is unfavorable for the activities of the soil bacteria concerned in ammonification, the soil fungi might prove to be an important compensating factor in maintaining fertility.—Z. N.

Influence of Barnyard Manure and Water upon the Bacterial Activities of the Soil. J. E. GREAVES AND E. G. CARTER. J. Agr. Res., 1916, **6**, 889–926.

This is a report of a fairly extensive investigation of the bacteriological effects of different quantities of manure and water added to soil. Plate counts are made on Lipman's "synthetic agar." Determinations of ammonifying and nitrifying powers of the soil are made by the tumbler method, while nitrogen-fixing powers are determined by adding the soil to Ashby's solution. Part of the work is done on potted soil, part on field soil. The detailed results are too many to give in a brief review; but in general the writers find a direct relationship between the bacterial count, the ammonifying powers, the nitrifying powers, and the crop produced. As a rule all these factors are increased with increasing amounts of manure, and with increasing moisture content up to at least 20 per cent.—H. J. C.

The Effect of Time and Depth of Cultivating a Wheat Seed Bed upon Bacterial Activity in the Soil. P. L. GAINEY. Soil Science, 1916, **2**, 193–204.

Call of Kansas presented a paper concerning the effects of different methods of preparing a seedbed for wheat upon yield, soil moisture, and nitrates. Gainey's article is concerned with the very marked effects of the various experimental methods of Call upon the accumulation of nitrates. Gainey determined from his own experimental evidence that the differences in nitrate content reported by Call cannot be attributed to a difference in the bacterial content. Some non-biological condition existing in certain plots under field conditions prevents the normal activity of the bacterial flora. Among the factors controlling bacterial activity, the available moisture probably plays a paramount rôle.—Z. N.

Azotobacter in Hawaiian Soils. P. S. BURGESS. Soil Science, 1916, **2**, 183–192.

Thirty soils from different localities on the four large islands of the Hawaiian group were examined as to their azotobacter content, and their abilities to fix nitrogen in mannit solutions. Only five of the soils failed to show azotobacter growth in solutions. Four different forms of azotobacter were isolated in pure cultures, described and their abilities to fix nitrogen in solutions and in soils determined. A possible explanation of the introduction and distribution of azotobacter in these isolated island soils is given. With the introduction of commercial mixed and single element fertilizers, a fine soil is sometimes used as a "filler." Azotobacter species may have been carried in the latter, as it is never sterilized. The imported organic fertilizers, legume seeds, etc., may also have been carriers of this bacillus.—Z. N.

The Effect of Some Manganese Salts on Ammonification and Nitrification. P. E. BROWN AND G. H. MINGES. Soil Science, 1916, **2**, 67–85.

The effect of the application to soils of manganese chloride, manganese sulfate, manganese nitrate and manganese oxide, upon ammonification and nitrification, is given, with the following conclusions:

If manganese salts in small quantities increase crop yields on a soil, that increase may be due, in part, at least, to a beneficial effect on ammonification and nitrification, with a consequently greater production of available plant food.

On the other hand, if manganese salts when applied to the soil restrict crop growth, that restriction may be due in part to a depression of bacterial activity. The amounts of manganese salts which may be applied to any one soil without danger of depressing ammonification and nitrification are exceedingly variable.—Z. N.

Environmental Factors Influencing the Activity of Soil Fungi. D. A. COLEMAN. Soil Science, 1916, **2**, 1–65.

The type of soil as well as the quality of the organic matter were found to regulate the activities of the organisms used in the tests. From the standpoint of pure cultures, every organism will do best with a definite combination of soil and organic matter. As a general rule, vegetable matter of high quality was conducive to greatest activity.

Different species of fungi respond with a very wide divergence to the mechanical composition of the soil by which the oxygen supply is determined. Chemicals beneficial to one group or species may be detrimental to other groups of organisms, suggesting a possible alteration of group relations among the microbes in the soil. This is also true of the moisture content of the soil.

The organisms employed were observed to have a very narrow temperature range with an optimum of about 30°.—Z. N.

The Yield and Nitrogen Content of Soy Beans as Affected by Inoculation. J. G. LIPMAN AND A. W. BLAIR. Soil Science, 1916, **1**, 579–584.

Soy beans were used for this experiment as they are less likely to become inoculated spontaneously than other legumes which may be used in tests of the value of commercial cultures for soil inoculation. Moreover, the plants are rather hardy and may be made to grow without difficulty under a wide range of soil and climatic conditions.

Seven commercial cultures from different firms, and soy bean and cow pea soil were compared with uninoculated checks. Nitrogen accumulated was determined by the Kjeldahl method.

The authors conclude that the use of inoculating material may be very desirable in the growing of soy beans and perhaps other legumes. It appears that where the soil is lacking in the right type of *Ps. radi-*

cicola, inoculation is eminently desirable and that, even where the organisms are present in limited numbers, the addition of larger numbers may be profitable.

A marked difference in the quality of the different commercial cultures for soil inoculation was noted, as was also apparent with the inoculated soils employed. The variation was not greater with the soils than with the commercial cultures.—Z. N.

The Oxidation of Sulfur in Soils as a Means of Increasing the Availability of Mineral Phosphates. J. G. LIPMAN, H. C. McLEAN AND H. C. LINT. Soil Science, 1916, 1, 533–539.

Environmental conditions play an important rôle in the activities of sulfur oxidizing microörganisms. In soils containing sulfofying bacteria under favorable conditions for their development elementary sulfur is readily oxidized. Besides an abundant supply of oxygen, moisture and the amount and quality of the organic matter are factors of direct significance. Moreover, the numbers and physiological efficiency of the organisms themselves are always of prime importance.

A strong analogy is shown between nitrification and sulfofication. The oxidation of sulfur in soils by bacteria may lead to the accumulation of large quantities of sulfuric acid, which in turn readily reacts with basic substances, e.g., tri-calcic phosphate; this may then furnish available phosphoric acid to crops.

Compost heaps as well as cultivated fields may be so treated as to provide a congenial environment for sulfofying bacteria and thus may be utilized for the production of available phosphoric acid out of insoluble phosphates.—Z. N.

Bacteriological Studies of a Soil Subjected to Different Systems of Cropping for Twenty-five Years. P. L. GAINEY AND W. W. GIBBS. J. Agr. Res., 1916, 6, 953–975.

This is a report of investigations of various soil plats that had been under the same system of cropping for twenty-five years, some under continuous cropping, some in rotation. The tests made are: number of bacteria developing on plates, ammonifying and nitrifying powers. The writers conclude:

"The soil under continuous corn and wheat contains, in the absence of any additions of fertilizers or manure, relatively low numbers of bacteria. In the presence of manure, continuous corn and wheat soil contain relatively high numbers. . . . .

"The agricultural practices under study . . . . produced no appreciable effect upon the ability of the soil and its organic life to liberate ammonia from cottonseed meal.

"The ability of the soil complex to oxidize ammonia nitrogen to nitrate nitrogen has been materially altered by the methods under study. . . . . Continuous corn and wheat with no additions of manure or chemicals have brought about a relative low oxidizing

power in the soil complex. The addition of manure materially raises the oxidizing power, especially under continuous wheat and corn. The addition of commercial fertilizer brings about a condition similar to that of manure, though perhaps less marked."—H. J. C.

Studies on the Activity of Soil Protozoa. G. P. KOCH. Soil Science, 1916, **2**, 163–181.

The approximate time for protozoa to excyst when the cysts come in contact with free water was determined to be five to seven minutes.

Data are given in regard to the influence of moisture content, physical character of the soil and the presence of organic matter upon the activity of protozoa in the soil. About 0.33 gm. of soil was taken for daily examination for active protozoa. The type of soil determines whether the addition of organic matter encourages protozoan development. It was indicated that the destructive ability of soil protozoa (if they possess this power) would be present only for a limited period, namely, in the early stages of organic decomposition. Increasing the porosity and aeration of the soils by the addition of sand did not increase the number of motile protozoa.

With the soils used, moisture seemed to be the primary limiting factor which determined the presence or absence of active protozoa.

With one exception, no correlation between the presence of protozoa in the active condition and numbers of bacteria could be seen. Increased numbers of bacteria were observed irrespective of the presence or absence of living protozoa. Inasmuch as the numbers of protozoa in comparison with the bacterial numbers are so small, even in the presence of such abnormal quantities of organic matter as were used in these experiments, it hardly seems that they would be of very great importance in agricultural practice.—Z. N.

Soil Fungi and their Activities. S. A. WAKSMAN. Soil Science, 1916 **2**, 103–156.

The author has endeavored in this paper to ascertain: (1) What fungi are true soil organisms, i.e., occur continually and in most soils; (2) The part that these organisms play in the fertility of the soil.

The micro-flora of eight soils from various portions of the United States was studied, from which were isolated nineteen species belonging to the Phycomycetes, several to the Ascomycetes, including unidentified species of yeasts, and eighty species to the Fungi Imperfecti, all of which were included in thirty-one genera. The most common genera of fungi as to numbers and species found in the soils investigated are, in the order of their occurrence, as follows: *Penicillium, Mucor, Aspergillus, Irichoderma, Cladosporium, Fusarium, Cephalosporium, Rhizopus, Zygorhynchus, Acrostalagmus, Alternaria* and *Verticillium.* A hypothetical soil flora is given.

Physiological studies were made of types representative of fungus groups with regard to their nitrogen-fixing and ammonifying power,

their ability to digest starch and to decompose cellulose. The ammonifying and cellulose decomposing power of the fungi tested proved to be strong, the starch digesting and the nitrogen-fixing action to be weak or absent.

Cultivated and uncultivated soils do not differ distinctly in the species of their fungus flora, though each soil seems to have a more or less characteristic fungus flora. The numbers of fungi decrease rapidly with depth, so that at twelve to twenty inches below the surface very few fungi can be found, the largest numbers occurring within the upper four inches of soil. *Zygorhynchus Vuilleminii* was often the only organism present in sub soil.

Many pathogenic fungi have been isolated from the soil, a fact which leads one to think that they pass certain stages of their life history in the soil, or are able to live saprophytically in the soil and perhaps play a part in its fertility.—Z. N.

BACTERIOLOGY OF WATER AND SEWAGE

Some Aspects of Chlorination. Joseph Race. Jour. Am. W. W. Assn., 1916, **3**, 439; Can. Engr., 1916, **30**, 603–605.

In the use of liquid chlorin for sterilizing water thorough mechanical mixture is required. Tests at Ottawa show a saving in chlorin with higher bacterial removals. Data are given indicating that more chlorin is required for high color and when the temperature is low. Studies of the surviving types of *B. coli* did not indicate greater resistance than that possessed by the original culture.—L. P.

A Preliminary Report Upon Purification of Swimming Pools at the State University of Iowa. J. J. Hinman. Eng. and Contr., 1916, **46**, 135–138.

Two types of filters are in use, a pressure filter for the men's pool and a gravity type for the women's pool. Contract with the filter company guarantees an effluent which will conform to the government standard for water on interstate carriers. Out of tests on eighty-three consecutive days, with 1 cc. samples, only three presumptive tests for *B. coli* were secured on the women's pool and seven on the men's pool. Alum and liquid chlorine are used with the usual apparatus for their application. The author believes that the 37°C. count gives a better indication of the degree of purification than the 20°C. count.

F. W. T.

The Sanitary Control of Swimming Pools. Max Levine. Jour. Infect. Diseases, 1916, **18**, 293–306.

The author reviews the investigations on swimming pool disinfection and reports his own results involving the use of filtration, calcium hypochlorite, bleach, and copper sulfate. Continuous filtration effected a reduction of 60 per cent in the bacterial count, the results

on *B. coli* being better when the temperature of the water was below 23°C. But filtration alone was found insufficient to maintain a sanitary condition, and continuous disinfection with copper sulfate is recommended. The chemical should be added, at the rate of 1 part per million, three times per week, preferably just before the pool is opened. This method, together with an occasional sterilization with calcium hypochlorite keeps the pool in good sanitary condition for several months.—P. B. H.

CLASSIFICATION OF BACTERIA

Studies on the Paratyphoid-enteritidis Group. C. KRUMWIEDE, JR., J. S. PRATT AND L. A. KOHN. Jour. Med. Res., 1916, **34**, 355–358.

In a study of a series of cultures of the pathogenic types of the paratyphoid-enteritidis group the authors found that xylose was fermented by all of the types except paratyphoid A, and suggest this as a presumptive test for this group.—H. W. T.

A Study of the Grouping of the Meningococcus Strains. MIRIAM P. OLMSTEAD, PHOEBE L. DuBois, JOSEPHINE B. NEAL, AND ROSE SCHWEITZER. Collected Studies, Bureau of Laboratories, New York City, 1914–1915, **8**, 180.

By means of complement fixation the meningococcus may be clearly differentiated from allied organisms. A differentiation of individual meningococcus strains is possible by use of refined technique, but the relationship of strains is so close that it is difficult to obtain absolutely clear-cut and consistent results. Of the 29 strains studied, 14 seem to form one group and 8 another. Three are related to the first group but have acted so irregularly that they cannot be classed with it; 2 have shown a relationship with each other only and 2 have shown a relationship to no other strain.—T. G. H.

A Study of Gas-Production by Different Strains of Bacillus abortivo-equinus. E. S. GOOD AND S. S. CORBETT. Jour. Infect. Diseases, 1916, **18**, 586–595.

It was ascertained that this organism produced approximately 2 per cent gas in lactose in 80 per cent of 116 trials; and slightly less than 2 per cent in sucrose in 50 per cent of 56 trials. Comparisons with the fermentation power of related organisms cause the authors to conclude that lactose and sucrose can be employed to advantage in differentiating *B. abortivo-equinus* from the colon bacillus, while dulcite can be used to differentiate it from the members of Subgroup II of the colon-typhoid group, although absolute proof must depend upon other characteristics.—P. B. H.

A Non-Gas-Producing Strain of the Hog-Cholera Bacillus Isolated from an Old Laboratory Culture. C. TENBROECK. Jour. Exp. Med., 1916, **24**, 213–222.

In a stock culture of the hog-cholera bacillus, which was passed through a series of rabbits 14 years ago, an organism was found that differs from the original culture in that it fails to form gas from the carbohydrates that are usually attacked by this organism, while acid formation persists. This new strain is agglutinated by an anti-hog-cholera bacillus serum and produces in rabbits and mice a disease similar to that caused by the typical cultures. The failure to form gas has persisted over a period of 18 months and all attempts to cause the strain to revert to the original condition have failed. It resembles in many respects *Bacillus typhi* and it may be that some of the so-called typhoid cultures that are not agglutinated by antityphoid serum are non-gas-producing paratyphoids. Attempts to produce a similar change in a more recently isolated culture of the hog-cholera bacillus by means of animal passages and changes in the environment have been negative.—B. W.

IMMUNOLOGY

Report of the Committee on Standard Methods of Preparing Diphtheria Antitoxin. Amer. Jour. Public Health, 1916, **6**, 751–752.

A supplementary report to the one made in 1911.—D. G.

The Wassermann Reaction in Two Hundred and Fifty-one Tuberculous Dispensary Cases. W. R. JONES. Med. Record, 1916, **90**, 418–419.

Of 251 cases examined in the tuberculosis clinic, 73 gave a positive, and 178 a negative Wassermann.—M. W. C.

Autotherapy in Poliomyelitis. C. H. DUNCAN. New York Med. Jour., 1916, **104**, 342–343.

Report of a case of poliomyelitis treated by the hypodermic injection of the spinal fluid removed from the patient.—M. W. C.

A Note on the Serum Treatment of Poliomyelitis (Infantile Paralysis). S. FLEXNER. Jour. A. M. A., 1916, **67**, 583–584.

A review of the work already reported from the Rockefeller Institute on the serum treatment of infected monkeys, and the work of Netter on the treatment of human poliomyelitis with the serum of persons completely recovered from the disease.—G. H. S.

Vaccine Therapy and Other Treatment in Acne Vulgaris and Furunculosis. H. H. Fox. Jour. A. M. A., 1916, **66**, 2064–2067.

From an analysis of 100 cases it would appear that the treatment of these conditions by vaccines, either autogeneous or stock, does not effect as high a percentage of cures as do other therapeutic measures.

G. H. S.

Inoculation against Typhoid in Maryland. F. W. HACHTEL AND H. W. STONER. Amer. Jour. Pub. Health, 1916, **6**, 703–706.

Of 14,795 residents of Maryland inoculated for from one to five years with typhoid vaccine but ten have since had the disease, which gives an attack rate of 6.75 per 10,000 persons. In contrast the attack rate for the state for a similar period (5 years) was 33.30 per 10,000 persons.

D. G.

Autolysis of Anaphylactic and Immune Tissues. W. H. MANWARING AND RUTH OPPENHEIMER. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 176.

The post-mortem autolysis of livers from normal, anaphylactic and immune guinea pigs was tested by determination of coagulable and non-coagulable nitrogen. Autolysis was increased in the immune livers.—W. J. M.

Universal Immunization. H. B. BARUCH. Medical Record, 1916, **90**, 372–373.

The author proposes that children should be injected at an early age with serum of adults who have recovered from scarlet fever, measles, and other infectious diseases. The author maintains that such serum contains antibodies and should therefore confer immunity upon the recipient.—M. W. C.

The Bacteriological Aspect of the Abderhalden Test. D. RIVAS AND A. C. BUCKLEY. Jour. Med. Res., 1916, **34**, 297–304.

The article is essentially a summary of recent opinions regarding the value of the Abderhalden reaction as a diagnostic test. On the theory that the presence of products of tryptic digestion may be indicated by the formation of indol by *B. coli* after a short incubation, the authors apply this test to a series of cases with negative results.

H. W. L.

The Effects of Exposure to Cold upon Experimental Infections of the Respiratory Tract. J. A. MILLER AND W. C. NOBLE. Jour. Exp. Med., 1916, **24**, 223–232.

By subjecting rabbits to sudden changes in temperature, from low to high, and from high to low, and then inoculating them by spraying cultures of *Bacillus bovisepticus* on the nasal mucous membrane, it was found that any marked change of temperature predisposed the rabbits to this infection, the severity of which varies with the amount of change, and that a change from low to high temperature has an even more marked effect than that from high to low.—B. W.

The Effect of Moderately High Atmospheric Temperatures upon the Formation of Agglutinins. C.-E. A. WINSLOW, JAMES ALEXANDER MILLER, AND W. C. NOBLE. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 194.

Rabbits kept at a temperature of 29° to 32°C. for five weeks and injected intraperitoneally twice a week with killed typhoid bacilli, produced agglutinins somewhat less powerful than those of the control animals kept at room temperature, 18° to 21°C.—W. J. M.

A Note on Experimental Nephropathy from Some Bacterial Poisons. J. L. STODDARD AND A. C. WOODS. Jour. Med. Res., 1916, **34**, 343–355.

Studying the effect of injections of bacterial poisons of the streptococci and staphylococci, in comparison with the protein split products of Vaughan, the authors find that these poisons have a special affinity for the epithelial cells of the kidney. Although somewhat similar, the bacterial poisons do not correspond completely in their pathological effect with those of the Vaughan poisons.—H. W. L.

Vaccines in Acute Infection. E. BONIME. Medical Record, 1916, **90**, 282–284.

To insure a successful use of bacterial vaccines, care must be taken that the bacteriological diagnosis of the etiological factor is correct, that the vaccine is carefully prepared and administered at the proper time and at suitable intervals, and that further growth of the causative organisms is prevented. Without these precautions, failures may occur in vaccine therapy which cannot be justly ascribed to the vaccine itself.—M. W. C.

A Bacteriological Study of Pyorrhoea Alveolaris and Apical Abscesses in Relation to Vaccine Therapy. CHARLES KRUMWIEDE, JR., AND JOSEPHINE S. PRATT. Collected Studies, Bureau of Laboratories, New York City, 1914–1915, **8**, 166.

In a preliminary study of mouth conditions, the great complexity of the flora found in pyorrhoea is shown. Vaccines made from a few types present would not influence the pyorrhoeal condition.

A study of the *Streptococcus viridans* group should be made, including as many strains as possible of known invasive capacity, as well as those from mucous membranes. Much of the vaccine therapy as now practised, rests on doubtful assumptions.—T. G. H.

The Cause, Treatment, and Prevention of Hay Fever. W. SCHEPPEGRELL. Medical Record, 1916, **90**, 95–98.

Among the effective methods of treatment are immunization with pollen extracts, the administration of autogenous vaccines, prepared from the bacteria found in the nasal secretion of the patient, and the use of calcium chloride and succinimide of mercury.

Particular emphasis is laid upon the necessity for preventive measures, especially the eradication of pollinating weeds.—M. W. C.

The Coexistence of Antibody and Antigen in the Body. B. S. DENZER. Jour. Infect. Diseases, 1916, **18,** 631–645.

Antigen and antibody persisted in both the cells and blood for three weeks after immunization of a guinea-pig with foreign serum. Antigen was demonstrable in the cells and in the blood for 17 days. Later it disappeared. Antibody was demonstrable in the cells from the ninth day and in the blood after the fourteenth day.—P. B. H.

The Action of Antiseptics in Pasteur Antirabic Emulsions. DANIEL W. POOR. Collected Studies, Bureau of Laboratories, New York City, 1914–1915, **8,** 191.

In an effort to obtain a substitute for glycerin in antirabic emulsions, several antiseptics were tried.

Chinosol in dilutions of 1: 10,000; 1: 15,000 and 1: 20,000 offered no advantages over other agents, which are non-proprietary and more easily obtainable. Dilutions of 1: 10,000 effected the strength of the rabies virus, and 1: 30,000 did not hold in check extraneous organisms. Camphor and menthol were of little value. Carbolic acid (0.2 per cent) gave the best result, the rabicidal effect being less than that of 20 per cent glycerin, and the antiseptic effect as good.—T. G. H.

An Epidemic of Whooping Cough Treated with Pertussis Stock Vaccine. MATTHIAS NICOLL, JR., AND PAUL LUTTINGER. Collected Studies, Bureau of Laboratories, New York City, 1914–1915, **8,** 86.

Out of 350 children who had been exposed to whooping cough, half were treated with pertussis vaccine. None of them took the disease. If all had been treated, freedom from disease probably would have been ascribed to the vaccine.

The possibility of immunization against pertussus is still an open question.—T. G. H.

A Case of Sensitization to Witte's Peptone. K. R. COLLINS. Proc. N. Y. Pathol. Soc., 1916, **16,** 46.

The patient was a laboratory worker who acquired a very marked sensitiveness to peptone, such that severe coryza, conjunctivitis and edema of the glottis would follow inhalation of minute quantities of it. He appeared not to be sensitized to other substances.—W. J. M.

Preliminary Studies of the Antigenic Properties of Different Strains of Bacillus Typhosus. SANFORD B. HOOKER. Proc. Soc. Exp. Biol. and Med., 1916, **13,** 139.

Serum cross-titrations with standardized antigens indicated a division of typhoid strains into three groups. Group I strains cross-fixed with all antigens. Those of Group II cross-fixed with each other

but not with antigens of Group I. Strains of Group I–A gave irregular results. The results suggest that a polyvalent vaccine should be used in typhoid prophylaxis.—W. J. M.

Characteristics of the Precipitation Reaction. RICHARD WEIL. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 200.

Chemically pure antigen unites with precipitin in proportions which are definite and constant, so that the Danyz-Dungern phenomenon cannot be demonstrated in the precipitation reaction when performed with pure reagents. The reaction is probably a quantitative chemical reaction and not comparable to the adsorption phenomena of mutually precipitating colloids.—W. J. M.

Immunity in Syphilis. H. ZINSSER. Jour. Lab. and Clin. Med., 1916, **1**, 785–802.

From clinical and experimental observations it does not seem probable that an infection of syphilis confers true immunity. Although the syphilitic subject acquires a definite resistance to reinoculation, which is most pronounced in the secondary stages of the disease, this resistance decreases during the tertiary stage and probably disappears entirely upon cure, so that recovery from syphilis leaves the patient as susceptible to infection as a normal individual. Recovery from the disease does not occur spontaneously and any apparent immunity to reinfection is an evidence of persistence of the disease in a latent form.—M. W. C.

Endothelial Opsonins. W. H. MANWARING AND HARRY C. COE. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 171.

When the blood-free liver of an immunized rabbit is perfused with a suspension of pneumococci in Ringer's solution, the bacteria are rapidly removed from the fluid and adhere to the capillary endothelium. Immune serum added to the suspension of bacteria gives rise to the same result when the suspension is perfused through normal livers. Suitable controls give negative results. The serum component resists a temperature of 60°C. for 30 minutes.

Extrahepatic capillaries fail to show similar phagocytic properties but the spleen and bone marrow have not been tested as yet.—W. J. M.

Specific Receptors of Fixed Tissues. W. H. MANWARING AND YOSHIO KUSAMA. Proc. Soc. Exp. Biol., and Med., 1916, **13**, 172.

Ringer's solution containing 1 per cent goat serum, repeatedly perfused through blood-free liver of normal, anaphylactic or immune rabbits shows no diminution in amount of serum, that can be detected by titration with specific precipitating serum. Analogous results are obtained if normal anaphylactic or immune rabbit blood is added to the perfusion fluid. The results furnish no evidence of the existence of specific receptor apparatus in rabbit livers.—W. J. M.

Hepatic Bacteriolysins. W. H. MANWARING AND HARRY C. COE. (*Preliminary Report*). Proc. Soc. Exp. Biol. and Med., 1916, **13**, 177.

If pneumococci are deposited by perfusion methods in the liver of a normal rabbit in the presence of normal rabbit blood and the liver then incubated at 37°C., the bacteria multiply and overgrow the liver after six hours. If the analogous experiment is tried with liver and blood of an immune rabbit a gradual decrease in the deposited pneumococci is observed. The bacteria in the larger vessels, not in contact with parenchyma, are not destroyed. There is apparently an hepatic mechanism in the immune animal for the destruction of microörganisms.—W. J. M.

Protein Absorption by Blood Corpuscles. W. H. MANWARING AND YOSHIO KUSAMA. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 173.

Freshly drawn defibrinated rabbit's blood added to a 1 per cent solution of goat's serum and incubated for one hour shows only 25 per cent of the goat protein remaining in the fluid portion. If the serum and corpuscles are now separated and allowed to undergo independent autolysis (10 hours at 37°C.) a distinct restoration of the goat protein occurs in both, but is more pronounced in the corpuscle fraction. Analogous results may be obtained *in vivo*, indicating that parenterally introduced proteins are absorbed in a large measure by the circulating blood corpuscles.—W. J. M.

Analysis of the Anaphylactic and Immune Reactions by Means of the Isolated Mammalian Heart. W. H. MANWARING, ARTHUR R. MEINHARD AND HELEN L. DENHART. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 175.

The heart of a rabbit sensitized to goat serum, tested by perfusion with 7 to 10 per cent goat serum is more resistant than a normal heart. Hearts of rabbits sensitized or immunized by repeated injections are still more resistant. Normal rabbit serum, corpuscles or defibrinated blood, added to the perfusion fluid decreases its toxicity. Anaphylactic rabbit serum similarly added increases the toxicity. The active principle responsible for this effect is destroyed by heating to 60°C. for 30 minutes and is not restored by the addition of fresh normal serum, and is therefore not complement and probably not precipitin. Such inactivated anaphylactic sera are strongly antitoxic.

—W. J. M.

A Method of Producing Antigen for Complement-Fixation in Tuberculosis. H. R. MILLER AND HANS ZINSSER. Proc. N. Y. Pathol. Soc., 1916, **16**, 28.

A weighed amount of bacillary substance is ground up with salt and subsequently suspended in distilled water sufficient to give isotonicity. Such antigen is not anticomplementary in quantities of 1.0

cc. and has fixed in quantities as low as 0.02 cc. Sera from 190 patients have been tested and positive reactions were obtained in the 89 actively tuberculous cases while 93 clinically negative cases gave no fixation.—W. J. M.

Complement Fixation in Tuberculosis. H. R. MILLER AND HANS ZINSSER. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 134.

The antigen is made by grinding 0.020 gram of moist tubercle bacilli with 0.090 gram salt for one hour, then adding 10 cc. of distilled water. The results in 602 cases are reported. Out of 226 patients with clinical diagnosis of tuberculosis, 223 gave positive complement fixation. In 88 cases of arrested tuberculosis, the reaction was negative in 54, weak in 21 and positive in 13. Of 140 doubtful cases, 32, gave positive fixation and in some of these 32 a diagnosis of tuberculosis was subsequently made. Forty-five positive Wassermann sera were tested, 2 only giving a positive fixation with the tubercle antigen. One of these two patients was shown to have tuberculous peritonitis. The fixation seems to be positive only in active tuberculosis.—W. J. M.

A Review of the Complement Fixation Test in Tuberculosis. H. A. MILLER. Jour. Lab. and Clin. Med., 1916, **1**, 816–822.

The complement fixation test in tuberculosis has given fair results with a variety of antigens. Bacillary emulsions, tuberculins and extracts of bacilli are all available antigens. Particularly successful results have been obtained with the antigen of Miller and Zinsser. This is prepared by triturating living or dead bacilli with dry crystals of ordinary table salt, then adding distilled water up to isotonicity. This antigen is almost invariably positive with active cases, negative in arrested cases, and gives no cross fixation with luetic sera.—M. W. C.

A Modification of Römer's Intracutaneous Method for the Determination of Small Amounts of Diphtheria Antitoxin in Blood Sera. ABRAHAM ZINGHER. Proc. N. Y. Pathol. Soc., 1916, **16**, 49.

A standard, well-ripened toxin is freshly diluted with salt solution so that 1 cc. represents $\frac{1}{100}$ L+ dose. The serum to be tested is used undiluted and in dilutions of 1: 10, 1: 100, 1: 1000 and 1: 10,000. Of each serum dilution, 0.2 cc. is added to 0.2, 0.4, 1.0 and 2.0 cc. of the diluted toxin in four tubes and salt solution, 0.0, 0.2, 0.8 and 1.8 cc., is added to the respective mixtures, which are allowed to stand 30 minutes before being injected. The injections are made intracutaneously into the abdomen of guinea pigs, four widely separated injections to each animal, the dose being 0.2 cc. in each instance. The local appearance of the skin is recorded daily for four days. As little as $\frac{1}{500}$ unit of antitoxin in a serum can be estimated with a fair degree of accuracy.—W. J. M.

Gonorrhea and Its Complications. A. HYMAN. N. Y. Med. Jour., 1916, **104**, 308–309.

Twenty-five cases of gonorrhea were treated with the vaccine of Nicolle and Blaizot. Injections were given intramuscularly or intravenously for 6 to 8 treatments. The dose amounted to 3,000,000,000 bacteria and was not increased. Acute cases received injections every day or every other day, chronic cases every two to four days. No local therapy was employed.

Although 28 per cent of the cases were definitely cured by the vaccine, the results in general were inconstant. It had no effect upon cases of uncomplicated acute and chronic urethritis. Epididymitis was but slightly improved. Chronic prostatis responded most favorably to the treatment and gonorrheal rheumatism improved greatly in a few cases.—M. W. C.

Toluol: Its Value in the Sterilization of Vaccines Made from Nonsporogenous Gram Negative Bacteria. L. D. BRISTOL. New York Med. Jour., 1916, **104**, 360–361.

Toluol is advocated as an agent for killing vaccines. It does not impair the immunizing power of the vaccine as does heat or strong germicidal chemicals.

Agar slant growth is covered with toluol and allowed to remain for a length of time necessary to kill. Most Gram negative nonsporogenous bacteria will be killed in 24 hours. The toluol is then poured off and the cultures returned to the incubator to hasten the complete evaporation of the toluol. Sterility tests are made and the growth is suspended in salt solution, counted, and employed as a vaccine.

The staining characteristics of the organisms are retained if the contact with toluol has not been too long.—M. W. C.

Immunologic Studies on Hodgkins Disease. J. J. MOORE. Jour. Infect. Diseases, 1916, **18**, 569–585.

An attempt to ascertain whether an immune serum could be produced for this disease. Horses were immunized by intravenous inoculation of bacilli isolated from lymph glands. This serum was found to fix complement and to cause marked agglutination. Complement fixation tests made with serum of patients gave in no case inhibition of hemolysis with mixed antigens and vaccination with these organisms failed to increase complement-binding antibodies. Agglutination tests were also negative. Similar negative results were observed in the case of sera from cases of lymphosarcoma, lymphatic leukemia, chronic arthritis and tuberculosis.—P. B. H.

Bonime's Modification of Koch's Treatment of Tuberculosis. R. C. NEWTON. Med. Rec., 1916, **90**, 320–324.

Bonime's method of treating tuberculosis consists in the frequent administration by injection of minute quantities of tuberculin in gradually increasing doses. The principle is to provide a stimulation

of antibody production without causing an overstimulation, resulting in the liberation of large amounts of toxic products.

The initial dose consists of 0.1 cc. of a 1:10,000 dilution of O. T. Doses are repeated and increased in arithmetical progression, temperature conditions controlling the advance, until the patient can receive 0.2 cc. of pure O. T. without a reaction. Tuberculin B. E. is then employed and the immunization continued, doses being given at longer intervals.

When a patient can take 0.1 cc. of pure O. T. four times in a year without a reaction, he is presumably immune from infection with tuberculosis.—M. W. C.

Sputum Cultures with Subsequent Complement Fixation Control. W. W. WILLIAMS AND WARD BURDICK. Interstate Med. Jour., 1916, **23**, 508–512.

The technique described by the authors deals with infections other than tubercular. The mouth of the patient is thoroughly cleansed. The specimen is then raised from the deep pharyngeal region. The mass of sputum is washed in sterile saline solution and the mucoid fibers smeared over human blood agar plates. If a vaccine is desired, the growth is washed off the plates with salt solution containing 0.3 per cent tricresol. The suspension is standardized and diluted so that the tricresol is only sufficient for purposes of preservation.

The patient's serum is tested by the complement fixation test, using the autogenous antigen and stock antigens of organisms which might be expected to cause the inflammation. The autogenous antigen seldom fails to give a positive reaction and the corresponding stock antigen generally gives positive results, except in the case of streptococci. This confirmatory test is a distinct advantage in vaccine therapy.—G. H. R.

Complement-Fixation in Pulmonary Tuberculosis. A. MEYER. Medical Record, 1916, **90**, 232–235.

Report is given of the results of complement fixation tests with tubercular sera and a new antigen.

The antigen is polyvalent and is made from young cultures of human strains by grinding 20 mgm. of moist tubercle bacilli with 90 mgm. of salt for an hour, adding distilled water to isotonicity, and separating heavier clumps by allowing the suspension to stand a few minutes after shaking. This antigen is not anticomplementary in 1 cc. quantities, and fixes positive sera in 0.02 cc.

The test is carried out with one-half the original Wassermann quantities, using 2 units of amboceptor and 2 of complement. The anti-sheep rabbit hemolytic system is used.

Of the cases tested 96 per cent of those with positive sputum reacted positively; 93 per cent of doubtful cases gave positive results, and these results were later substantiated by clinical or skiagraphic

findings. In 22 cases of various diseases which gave negative reactions, 86 per cent were proved to be non-tuberculous.

With this antigen complement fixation in tuberculosis is considered of as much value as the Wassermann test for syphilis.—M. W. C.

Studies on Intradermal Sensitization, I. Intradermal Reactions to Emulsions of Normal and Pathologic Skin. John H. Stokes. Jour. Infect. Diseases, 1916, **18**, 403–414.

The present work is an effort to explain certain conflicting observations on cutaneous sensitization. It is reported that normal skin reacts to intradermal injection of skin emulsions in a definite though variable manner, comparable with the papular luetin reaction, and a positive reaction may be judged by the same criteria. No specific character could be established in normal persons for the response toward their own as compared with other's skin emulsions, and no evidence of Sellei's "homoasthesia" was found. A suspension of proteins from blood clots gave rise to more transient reactions. In a single test positive results were obtained from the use of a boiled (Kozilek's) emulsion. The emulsion possessed no antigenic properties, and there was no evidence of active anaphylaxis to the proteins. The attempt to immunize guinea-pigs passively by means of serum from the donors of the emulsion resulted negatively.—P. B. H.

Studies in Intradermal Sensitization, II. An Intradermal Reaction to Agar and an Interpretation of Intradermal Reactions. John H. Stokes. Jour. Infect. Diseases, 1916, **18**, 415–436.

The author presents the results of his study of reaction to agar and enters upon a general discussion of the mechanism of intradermal reactions in general, with special reference to anaphylatoxin formations. He concludes that: Reactions to luetin, pallidin, agar, iodid, placental tissues and skin emulsions, may be regarded as in part due to the introduction of antiferment adsorbents, the activity of which uncovers the ferments normally present. These proteases split up the proteins of the subject with the formation of anaphylatoxins producing focal necrosis. The course of reaction is determined by the success or failure of the body cells in the effort to restore the antienzyme-protease balance at the site of the injection. Systemic symptoms may be due to the escape of toxins into the circulation. Such reactions may be considered as non-specific, conceivably due to the action of the patient's own enzymes on his own proteins, not to a specific reaction between the substance injected and a specific amboceptor in the blood. The author suggests that reaction or non-reaction may find its solution in investigations of variability in the ferment balance, either locally or in the body at large.—P. B. H.

Studies on Treponema Pallidum and Syphilis. HANS ZINSSER, J. G.
    HOPKINS, AND MALCOLM MCBURNEY. Proc. N. Y. Pathol. Soc.,
    1916, **16**, 15.

Five strains have been kept alive through ten or more generations
in rabbits. Four of these were newly isolated and the fifth was the
strain of Dr. Nichols. Apparent fluctuations in virulence are believed
to depend upon the character of the lesion from which the virus is
taken for inoculation, the size of the testes of the rabbit to be inocu-
lated, the thoroughness of maceration of the material before inocula-
tion, the delay before inoculation and the exact site at which the in-
jected material is placed. The apparent fluctuations are therefore
not considered as real alterations in virulence. Variation in thickness
of the spiral occurred in the different strains and was not characteristic
of any one in particular.

The agglutination tests failed to distinguish *Treponema pallidum*
clearly from other similar organisms, notably *Tr. calligyrum,* even when
absorption methods were employed.

Normal rabbit serum was found to be treponemacidal when consid-
erable amounts were used. Immune serum was treponemacidal in
one-tenth the quantity. The germicidal property was destroyed by
heating the serum to 56°C. for half an hour.

Agglutination tests failed to distinguish syphilitic human serum
from that of patients free from syphilis.

Agglutination occurred more readily in treponemata which have
been grown in artificial culture a long time.

The preparation from cultures of a specific antigen for the Wasser-
mann test is being studied.—W. J. M.

*Animal Experiments upon the Acquirement of Active Immunity by
    Treatment with Von Ruck's Vaccine against Tuberculosis.* F. J.
    CLEMENGER AND F. C. MARTLEY. Medical Record, 1916, **90**, 135–
    142.

Experiments were conducted to determine whether the use of von
Ruck's vaccine was followed by an active immunity to tuberculosis.

Clinical use of the vaccines in cases of lymph gland affections re-
sulted in no permanent changes in the local lesions, but in four out
of six cases there was improvement in the general condition. The sug-
gestion is made that the insusceptibility to treatment of the local
conditions was due to the fact that owing to the minimum of circulation
in the affected tissue, no elaboration of immune bodies, however
marked, would be met by a local response.

Attempts to produce immune sera which would conform to a given
standard as expressed by the complement fixation test were partially
unsuccessful because of the prevalence and interfering action of
pseudotuberculosis.

Bactericidal experiments with tubercular human sera demonstrated
that animals which had received a mixture of serum and tubercle ba-

cilli were more resistant to infection than those in which salt solution or normal serum was substituted for the tubercular serum.

The results obtained after infecting pigs which had been actively immunized with von Ruck's vaccine were not entirely satisfactory. On the whole, however, it appeared that animals possessing an active immunity showed a greater resistance to infection than control animals.—M. W. C.

LABORATORY TECHNIQUE

Culture Media for Paramecia and Euglena. R. M. STRONG. Science, 1916, **44**, 238.

The author describes a simple and convenient medium for the abundant production of *Paramecia* and *Euglena* for class work.—C. M. H.

Another Use of the Double Plate Method. W. D. FROST AND FREDA M. BACHMANN. Science, 1916, **44**, 433.

The senior author justly insists upon his priority in the use of the double plate method, introduced by him for the study of antagonism towards the *Bacillus typhi* in 1904. In the present work on antiseptic values of certain spices and condiments, he describes a modification of his method, made by substituting semi-discs of muslin for glass rods in the petri dishes. When the plain agar has solidified in one-half of the plate, the cloth is removed and the agar containing the condiment is poured into the other half.—C. M. H.

Studies on Laboratory Media. JANE L. BERRY. Collected Studies, Bureau of Laboratories, New York City, 1914–1915, **8**, 288.

A medium containing the usual amount of agar and one-twelfth the amount of meat-extract, peptone and salt gave good results in milk plates, with much reduced cost. Still better results for milk work were given by agar made with a one to fifteen dilution of Hottinger's stock broth. This is also good for carrying stock cultures.

Agar which has been used once for cultivation of various organisms can be melted, poured together, titrated, sterilized, and used over again, especially where large numbers of bacteria are desired as in vaccine work.

Tests made on several American-made peptones compared favorably with Witte's peptone.—T. G. H.

A New Culture Medium for the Isolation of Bacillus Typhosus from Stools. J. E. HOLT-HARRIS AND OSCAR TEAGUE. Jour. Infect. Diseases, 1916, **18**, 596–600.

The authors point out that the chief difficulty in the use of the Endo plate is that colonies of *B. coli* may redden the entire plate and thus obscure typhoid colonies. They recommend a medium composed of nutrient agar containing peptone, sodium chloride, Liebig's meat extract, sucrose, lactose, eosin and methylene blue. On such

media colonies of *B. coli* are black, while the colonies of *B. typhi* are colorless. Moreover the colon colonies do not affect the media lying between the colonies. Other advantages are mentioned.
—P. B. H.

The Requirements of the Gonococcus for Its Natural and Artificial Growth. L. D. BRISTOL. Medical Record, 1916, **90**, 63–65.

From a theoretical standpoint it would appear that the gonococcus requires for its growth human glycoproteins, especially human mucins and related compounds. This conclusion is drawn from a study of the character of the proteins upon which the gonococcus grows in the human body.

Additional evidence that conjugated proteins are essential to the growth of the gonococcus is found in the fact that while the gonococcus will not multiply in the ordinary culture media, containing simple animal proteins, its best growth is obtained upon media containing human fluids, such as ascitic, cystic, or hydrocele fluids, blood serum or urine, in all of which mucoid substances of the conjugated protein group are found.—M. W. C.

Limitations to the Cultivation of Mammalian Tissues in Vitro. R. A. LAMBERT. Proc. N. Y. Pathol. Soc., 1916, **16**, 63.

Connective tissue is the only mammalian tissue which can be cultivated in vitro for any considerable period. Transplantable tumors show active growth only for a short time. Human malignant tumors have not been cultivated successfully although human connective tissue cells and wandering cells show marked activity in cultures.

The limited growth of tumor cells may be referable to several factors, mechanical injury, accumulation of metabolic products, presence of inhibiting antibodies and nutritional disturbances. Probably several factors are jointly responsible.—W. J. M.

MEDICAL BACTERIOLOGY

Case of Mastoiditis Complicated by Purulent Cerebrospinal Meningitis; Operation and Recovery. W. H. HUNTINGTON. Jour. A. M. A., 1916, **67**, 201–202.

Report of a case.

Friedländer's bacillus was the only organism found in the spinal fluid.—G. H. S.

Syphilis and Tuberculosis in the Same Lung. R. A. KEILTY. New York Med. Jour., 1916, **104**, 252–253.

Report of a case in which the tubercle bacillus and *Treponema pallidum* were demonstrable in the lung tissue.—M. W. C.

The Bulgarian Bacillus in the Treatment of Vulvovaginitis. M. B.
COHEN. Jour. Lab. and Clin. Med., 1916, **1,** 757–759.

Vulvovaginitis cannot be treated to advantage by means of cultures of *Bacillus bulgaricus*, since this organism does not thrive in the human vagina.—M. W. C.

The Types of Pneumococci in Tuberculous Sputum. HAROLD W.
LYALL. Collected Studies, Bureau of Laboratories, New York City,
1914–1915, **8,** 176.

Pneumococci were isolated from the sputum of 9 out of 25 tuberculous patients. The pneumococci were all of the type found in normal mouths.—T. G. H.

Tuberculosis of the Middle Ear. H. B. GRAHAM. Annals of Otology,
Rhinology and Laryngology, 1916, **25,** 105–118.

The author gives histories of nine cases.

Diagnosis was made by finding tubercle bacilli in microscopic preparations, after animal inoculation or in sections showing the pathological process.—C. P. B.

Gastro-Intestinal Findings in Acne Vulgaris. L. W. KETRON AND J.
H. KING. Jour. A. M. A., 1916, **67,** 671–675.

While it is conceded that the acne bacillus is the direct cause of acne vulgaris, an analysis of 30 cases suggests that gastro-intestinal derangements form an important predisposing factor.—G. H. S.

Focal Infection in Relation to Certain Dermatoses. M. L. RAVITCH.
Jour. A. M. A., 1916, **67,** 430–431.

Case reports are presented showing that many types of dermatoses may be due to focal infection. The removal of the infected part, teeth, tonsils, appendix, or of a streptococcic throat infection, resulted in cure.—G. H. S.

The Treatment of Chronic Colon Bacillus Pyelitis by Pelvic Lavage. H.
L. KRETSCHMER AND F. W. GAARDE. Jour. A. M. A., 1916, **66,**
2052–2053.

Of 14 cases of chronic colon bacillus pyelitis 11 bacteriologic cures were obtained by means of pelvic lavage employing silver nitrate in 1 per cent solution.

Treatments, at intervals of 5 or 6 days and numbering from 1 to 8, were required to free the urine of bacteria.—G. H. S.

Laboratory Facts in Poliomyelitis. S. R. KLEIN. New York Med.
Jour., 1916, **104,** 219–220.

An examination of about 400 cases of infantile paralysis showed that the blood and urine were uniformly sterile. The micrococcus of Weichselbaum was found in considerable numbers in the spinal fluid. No other organisms were demonstrable.—M. W. C.

Vincent's Bacillus in the Cervix. G. McConnell. N. Y. Med. Jour., 1916, **104**, 300–301.

A case report. The fusiform bacillus and the spirillum, so constantly present in cases of Vincent's angina, were found in almost pure culture in a smear prepared from an edematous cervix. A severe sore throat was coincident with the vaginal disturbance.—M. W. C.

Is Bact. Abortus (Bang) Pathogenic for Human Beings? L. H. Cooledge. Jour. Med. Res., 1916, **34**, 459–467.

No proof is offered that *Bact. abortus* is directly pathogenic for human beings. Adults drinking large quantities of milk from infected cows show, by the complement fixation test, the presence of antibodies in their blood. The authors believe this to be a passive immunity due to absorption of antibodies from the infected milk.

H. W. L.

A Bacteriologic Study of the Causes of Some Stillbirths. J. B. DeLee. Jour. A. M. A., 1916, **67**, 344–345.

Several case reports are presented. Pure cultures of *Streptococcus viridans*, the pneumococcus and an anaerobic nonhemolytic streptococcus were isolated from the organs of the fetus. The author suggests that many cases of so-called "habitual abortion" and repeated "premature labor after viability and before term" may be explained by bacterial infections.—G. H. S.

The Pathogenesis of Psoriasis. A. H. Cook. New York Med. Jour. 1916, **104**, 255–257.

From a study of ten cases it seems probable that psoriasis is due to infections with staphylococci and streptococci. This conclusion is based upon the fact that removal of the infections coexistent with psoriasis frequently resulted in recovery.—M. W. C.

The Etiology and Pathology of Otitic Cerebellar Abscess. Isidore Friesner. Annals of Otology, Rhinology and Laryngology. 1916, **25**, 92–104.

In the eighty-six cases collected the infectious agent is only stated eighteen times. *Streptococcus* was found eight times, *Staphylococcus* once. Vincent's spirillum and bacillus are mentioned. The author quotes Michaelsen's series in which the infecting agent was not found in pure culture even once.—C. P. B.

The Microscope in Dermatology. O. L. Levin. New York Med. Jour., 1916, **104**, 117–120.

A description of the use of the microscope in the diagnosis of the more common diseases of the skin as well as anthrax, glanders, blastomycosis, leprosy, sporotrichosis, actinomycosis, mycetoma, filarial elephantiasis, Dhobie itch, tinea imbricata, erythrasma, pinea, and diseases of the hair.—M. W. C.

Connellan-King Diplococcus. Infection of the Tonsil. J. J. KING. New York Med. Jour., 1916, **104**, 120–121.

From observation of 100 cases of septic arthritis, it appears that every case is caused by a focus of infection, which is most frequently situated in the tonsils. The causative organism has many times been found to be a Gram negative diplococcus, called the Connellan-King diplococcus. The administration of an autogenous vaccine, followed by the removal of the tonsils by enucleation constitutes a successful method of treatment.—M. W. C.

Studies on the Cultivation of the Rabies Virus. CAROLINE R. GURLEY AND CHARLOTTE C. VAN WINKLE. Collected Studies, Bureau of Laboratories, New York City, 1914–1915, **8**, 383.

Using the methods described by Noguchi, work was undertaken to determine with what regularity the cultivation of rabic virus could be obtained.

No evidence was found of the multiplication of the virus. The Negri-like bodies found by Noguchi were very rare and not definite in the experience of these authors. What may have been the same were once seen in a control tube of ascitic fluid of the kidney.

T. G. H.

Gas Bacillus Infection with Remarks on Gastric Ulcer. RANDOLPH WEST AND MARY E. STEWART. Proc. N. Y. Pathol. Soc., 1916, **16**, 30.

Filtrates of anerobic broth cultures of *B. welchii* killed guinea pigs, but not after neutralization of the acid. Acid solutions of equivalent strength were found to be toxic also. The guinea pigs frequently showed gastric ulcers and gastric ulcer without death of the animal was caused regularly by the intravenous injection of 4 to 4.5 cc. of 1 per cent acetic acid.—W. J. M.

Diphtheria in the First Year of Life. J. D. ROLLESTON. Amer. Jour. Diseases of Children, 1916, **12**, 47–52.

In an analysis of 2600 cases of diphtheria it was found that only 20 or less than 1 per cent were under one year of age. The mortality among these 20 cases was 45 per cent as compared with 7.3 per cent in the total. Three patients showed unmistakable signs of congenital syphilis and the author believes others were probably syphilitic. It is therefore concluded that syphilis is probably an important predisposing factor.—R. M. T.

Résumé on Infectious Diseases. ALBERT H. BEIFELD. Amer. Jour. Diseases of Children, 1916, **12**, 166–200.

A very complete and comprehensive résumé of the advances made in contagious diseases during the last few years is here given, together with a bibliography consisting of 300 references.

The following subjects are considered: hospital care, chicken pox, epidemic parotitis, measles, German measles, whooping cough, diphtheria and scarlet fever.—R. M. T.

Chronic General Infection with the Bacillus pyocyaneus. LEONARD FREEMAN. Annals of Surgery, 1916, 64, 195–202.

The case reported is one of extreme chronicity with typical neuralgic pains followed by paresis and muscular atrophy, and is of special interest because of the absence of the *B. pyocyaneus* in the blood and its presence in the bile; the absence of any discoverable point of infection, unless possibly the teeth; recovery through drainage of the gall-bladder and the use of an autogenous vaccine; the occurrence of cirrhosis of the liver (and its apparent disappearance since the recovery of the patient); the presence of ascites and pleural effusion, both of which promptly disappeared.—T. L. H.

The Bactericidal and Protozoacidal Activity of Emetin Hydrochloride in Vitro. JOHN A. KOLMER AND ALLEN J. SMITH. Jour. Infect. Diseases, 1916, 18, 247–265.

Emetin possesses bactericidal properties but prolonged contact is required. A 5 per cent solution failed to kill *B. typhi* in 15 minutes and a 2 per cent solution required 45 minutes to kill various bacteria from a case of pyorrhea. On the whole the effect was about equal to that of phenol in corresponding dilutions. Emetin proved to have some trypanocidal properties but these were less active than its amebacidal action.—P. B. H.

The Bactericidal and Protozoacidal Effect of Emetin Hydrochloride in Vivo. JOHN A. KOLMER AND ALLEN J. SMITH. Jour. Infect. Diseases, 1916, 18, 266–276.

In studying the bactericidal action of emetin on *Staph. aureus*, *B. tetanus* and *B. anthracis*, the authors found that the drug, administered intravenously, exerted a slight influence or none on the first named organism (infection in rabbits), and, when administered intraperitoneally to mice exerted no inhibitory action upon the other two. Administered intravenously to white rats, however, it exerted a slight germicidal influence upon *T. equiperdum* and *T. lewisi*. The authors conclude that the improvement or cure of pyorrhea alveolaris by emetin is to be attributed solely to its amebacidal action.—P. B. H.

Experimental Cholera-Carriers. OTTO SCHÖBL. Jour. Infect. Diseases, 1916, 18, 307–314.

The investigation was undertaken to ascertain whether animals could be made carriers of cholera vibrios. Inoculations were made into the gall-bladder, stomach, small intestine, blood stream and serous cavity; also by feeding. After direct inoculation into the gall-bladder, stomach and small intestine the organisms were found in the

alimentary canal, but intravesicular inoculation was more successful. The duration of infection in all cases was limited but appeared sufficient for therapeutic measures.—P. B. H.

The Localization of Streptococci in the Eye. E. E. Irons, E. V. L. Brown and W. H. Nadler. Jour. Infect. Diseases, 1916, **18**, 315.

A study of the invasive action of haemolytic streptococci from a case of iridocyclitis. Inoculated into rabbits the cultures produced iritis for 17 days after isolation, but showed a loss of the quality of localization. This could not be regained when once lost. The authors conclude that "the invasive power of an organism for special tissue may change within a short period of time during residence in the original host, during animal passage, and in culture, without pronounced or constant changes in cultural characteristics, or in general virulence for animals."—P. B. H.

Chronic Influenza in Pulmonary Tuberculosis. M. L. Hamblet and H. L. Barnes. Archives Int. Med., 1916, **18**, 313–316.

The purpose of the author's investigation was to determine the number of tuberculous patients infected with influenza bacilli and to determine whether the symptoms of patients diagnosed as having pulmonary tuberculosis could be caused by a chronic influenza infection.

Examination for influenza bacilli was accomplished by cultural and microscopic methods. Ninety-three to 96 per cent of patients diagnosed as having both open and closed tuberculosis appeared not to harbor influenza bacilli. The sputum of twenty patients in whom the diagnosis of tuberculosis was doubtful was negative for influenza bacilli.—G. H. R.

The Examination of the Urine and Feces of Suspect Typhoid Carriers with a Report on Elaterin Catharsis. F. O. Tonney, F. C. Caldwell and P. J. Griffin. Jour. Infect. Diseases, 1916, **18**, 239–246.

The writers report the following results of the examination of 290 specimens of urine and 298 specimens of feces: The use of elaterin catharsis is of assistance in detection of typhoid bacteria, and at the same time raises the value of a negative test. Endo plates were found most satisfactory. Lactose-peptone-bile was found to inhibit typhoid growth. The infectivity of typhoid carriers, in the absence of diarrhea is probably negligible. Typhoid bacteria were not found in the urine after administration of hexamethylenamin.—P. B. H.

A Contribution to the Chemotherapy of Tuberculosis, First Experimental Report. G. Koga. Jour. Exp. Med., 1916, **24**, 107–147.

Inspired by Koch's observation on the remarkable germicidal action of potassium auricyanide on the tubercle bacillus *in vitro*, Koga prepared a compound of copper and cyanide (details unfortunately not given) and studied its action on experimental tuberculosis in the guinea

pig. The results following the intravenous injection of this salt ("cyanocuprol") are summarized as follows: The effect of a single injection upon the lesions is either negative or inconspicuous. But after repeated injections of the preparation the congestion and leucocytic infiltration about the lesions are markedly decreased, the cheesy material resulting from degeneration of the lesions and other degeneration products are in process of absorption, and young connective tissue is being actively produced in the periphery. While these changes are taking place the number of bacilli is also being reduced until finally they can no longer be detected on microscopic examination. Whether the preparation brings about these results directly by killing the bacilli or indirectly by favoring the healing process of the body, it has power to inhibit the growth of or annihilate entirely the bacilli *in vitro*.—B. W.

A Contribution to the Chemotherapy of Tuberculosis. First Clinical Report. G. KOGA. Jour. Exp. Med., 1916, **24,** 149–186.

The author concludes, from a study of sixty-three cases, that cyanocuprol given intravenously to tuberculous individuals, greatly improves or apparently cures pulmonary and surgical tuberculosis in the first and second stage, and it seems also to produce beneficial effects upon the disease in the third stage. The duration of these beneficial effects is still to be established.—B. W.

The Treatment of Tuberculosis with Cyanocuprol. M. OTANI. Jour. Exp. Med., 1916, **2,** 187–206.

The paper deals with the clinical treatment of tuberculous patients with "cyanocuprol." The author claims marked efficacy for the treatment and gives directions for dosage, intervals for injection, precautions and contraindications.—B. W.

The Treatment of Leprosy with Cyanocuprol. R. TAKANO. Jour. Exp. Med., 1916, **24,** 207–211.

A brief report of treatment of leprosy with cyanocuprol which appears to be attended with beneficial results.—B. W.

Elective Localization of Bacteria in Diseases of the Nervous System. E. C. ROSENOW. Jour. A. M. A., 1916, **67,** 662–665.

A study of the possible etiologic relationship of focal infection to diseases of the nervous system.

Three strains of organisms, one staphylococcus and two green-producing streptococci, isolated from tonsils or teeth in cases of multiple sclerosis produced lesions of the spinal cord in 58 per cent of the animals inoculated.

A staphylococcus was isolated from a typical case of sporadic anterior poliomyelitis, which caused lesions of the spinal cord in 78 per cent of the animals injected.

Bacteria isolated from pyorrheal pockets and tonsils of a case of transverse myelitis caused lesions of the meninges in 50 per cent, and of the spinal cord, in 66 per cent of the inoculated.

Streptococci isolated from cases of brachial, intercostal and post-herpetic neuralgia produced lesions of the posterior roots in 83 per cent.

The pneumococcus isolated from multiple neuritis caused lesions of the peripheral nerves in 79 per cent.

Such elective localization warrants the conclusion that the lesions in the infected patients were due to an infection from a focal source.

G. H. S.

The Natural Resistance of the Pigeon to the Pneumococcus. PRESTON KYES. Jour. Infect. Diseases, 1916, **18**, 277–292, with one plate in color.

The author analyzes in detail the biologic factors which govern natural immunity in the pigeon to the pneumococcus. It was found that the organisms, whether injected intravenously or intraperitoneally, were rapidly withdrawn from the general circulation and localized in the liver and spleen. Here the ultimate localization and destruction was within a type of fixed phagocyte—the hemophage—having the normal function of destroying red blood cells. The destruction of the pneumococci by the hemophages is so rapid and complete as to constitute the determining factor in the production of natural immunity. The possible effect of temperature, of body fluids, of phagocytosis by wandering phagocytes (crystalloid acidophiles), and of opsonic factors are considered and ruled out as important factors in the final elimination of the pneumococci. Especial attention is directed to the function, in the spleen, of a double or triple layer of cells surrounding the endothelial intima which borders the lumen of certain smaller blood vessels. Through the agency of this zone of cells is brought about a mechanical filtration (interstitial) of the organisms which are eventually destroyed by the hemophages of the pulp cords. P. B. H.

Laboratory Aids in the Diagnosis of Poliomyelitis. J. B. NEAL. New York Med. Jour., 1916, **104**, 167–168.

One of the most interesting methods of laboratory diagnosis of poliomyelitis is the transmission of the disease to monkeys by inoculating them with the washings from the respiratory and alimentary mucous membranes. Sections of the brain from one monkey thus inoculated, showed a few globoid bodies similar to those described by Flexner and Noguchi.

Another method of some diagnostic value is the so-called neutralization test. Serum from a suspected case in the stage of recovery is mixed with a known fatal dose of an active virus. The mixture is incubated and injected intracerebrally into monkeys. In case poliomyelitis does not develop, there is indication that the virus has been neutralized. However, such neutralization may also be produced

through normal serum. Therefore, this method does not furnish conclusive evidence of poliomyelitis. Experiments with monkeys are too complicated and too costly for ordinary diagnostic use.

Examination of the spinal fluid offers the most trustworthy method of diagnosis. A spinal fluid increased in amount and showing a slight to moderate increase in albumin and globulin, a good reduction of Fehling's, and a varying cellular increase, mostly mononuclear, makes the diagnosis reasonably certain in fairly early cases of suspected poliomyelitis. A slightly cloudy fluid occurring very early in the disease must be differentiated from a similar fluid in an early purulent meningitis. Fluids from the cerebral or encephalitic type of poliomyelitis may sometimes be differentiated from fluids of tuberculous meningitis only by animal inoculation.—M. W. C.

Cultivation of the Organisms of Vaccinia, Variola, and Varicella. H.
    GREELEY. Medical Record, 1916, **90**, 265–271.

A minute, bipolar bacillus has been found in all specimens of vaccinia virus, and varicella and variola pustules. The organism occurs either in the form of a bacillus or in involution forms from which sporelike bodies escape. The organisms of vaccinia, variola, and varicella differ somewhat in size, but the general morphology is the same.

Cultural experiments upon the bacillus of vaccinia virus indicate growth upon hydrocele fluid, whether diluted in different proportions with physiological salt, distilled water, or bouillon, or when used alone. Media consisting of about 70 per cent hydrocele fluid and 30 per cent bouillon, and containing a lime water equivalent of 10 per cent were particularly suited to the growth of the organism. Fifty per cent bouillon in distilled water, saturated with 10 to 100 per cent calcium oxide was also a good medium. Both glycerin and glucose when added to the cultures exerted an unfavorable influence. Slants of Loeffler blood serum gave good growths when washed every day with one or two drops of limed bouillon mixture.

The virus of variola and also that of varicella grew under the same conditions as those which were found to be most favorable for vaccinia.

Agglutination tests showed specific results between variola sera and variola organisms, between vaccinia sera and vaccinia organisms. There was also agglutination between vaccinia sera and variola antigen. Varicella antigen reacted irregularly with all sera except those from varicella cases, with which it was uniformly negative.

In complement fixation the results were specific with variola sera and variola antigen, also with vaccinia sera and vaccinia antigen. Two vaccinia sera gave slightly positive reactions with variola antigen, but there was no cross reaction between variola sera and vaccinia antigen. Sera from cases of varicella gave negative results with varicella and variola antigens, but normal sera in almost every case reacted positively with varicella antigen.—M. W. C.

A Study of the Etiology of Chorea. JOHN LOVETT MORSE AND CLEVE-
LAND FLOYD. Amer. Jour. Diseases of Children, 1916, 12, 61–72.

Twenty-six cases of chorea were studied, of which twenty-one gave
no suggestive history of syphilis and only one gave a positive Wasser-
mann and one a doubtful reaction. This percentage is no higher
than that obtained among hospital children in general. It is therefore
concluded that syphilis probably plays no part in the etiology of chorea.
Seven of the patients or 37 per cent gave a history of rheumatism, six
of them had acute endocarditis and six chronic valvular lesions, a
total of 46 per cent. The tonsils were enlarged in eleven or 42 per cent
and had been removed in four; the teeth were carious in nineteen or
73 per cent, pyorrhea being present in two of these and pockets of
pus in three others.

Thirty-one blood cultures were made from the twenty-six cases
and the cerebro-spinal fluid was cultured twenty times in nineteen
cases. Loeffler's blood serum, neutral and acid milk, serum water
glucose, lactose and sucrose, glucose bouillon and at times hydrocele
fluid, alone and with agar were employed, both under aerobic and
anaerobic conditions. In five cases organisms were obtained. One
was a small gram negative "diphtheroid" bacillus, which grew very
poorly and failed to produce any effect upon rabbits. Diplococci were
found in the blood smears once but did not grow. In three cases
streptococci were grown in the original culture but in only one were
transplants successful. The authors think that these organisms were
probably alike. The one subcultured was injected into rabbits with
the production of endocarditis, arthritis and a congestion and round
cell infiltration of the pia over the cortex and extending into the con-
volutions. In one of the three rabbits in which the brain was exam-
ined smears from the cortex revealed streptococci.

The number of cells in the cerebro-spinal fluid taken from ten of the
patients was 2, 5, 7, 8, 10, 10, 10, 18, 24, and 25 respectively showing
a slight increase in 3 or 30 per cent.

The authors conclude that there is a definite relationship between
chorea and rheumatism, endocarditis and infections about the oral
cavity, and that their results suggest that a microörganism or group of
microörganisms may be the cause of chorea.—R. M. T.

PHYSIOLOGY OF BACTERIA

*The Destructive Effects of Light and Drying and Other Living-Room
Conditions Upon Diphtheria Bacilli, Streptococci, and Staphylococci.*
DANIEL W. POOR AND CHARLES P. FITZPATRICK. Collected Studies,
Bureau of Laboratories, New York City, 1914–1915, 8, 197.

Membranes from four cases of diphtheria, and mucus from one case,
with mucus from one case of scarlet fever were subjected to various
light and drying tests. When exposed to diffuse light, drying and
admixture of dust and other bacteria, diphtheria bacilli, staphylo-
cocci, and streptococci were rapidly reduced in numbers during the

first few days, after which there was a more gradual reduction. Streptococci seldom resisted drying and light longer than a week, nor diphtheria bacilli more than three weeks.

Ascitic broth cultures sometimes showed the presence of a few surviving diphtheria bacilli and streptococci after plate cultures had failed.

The reduction in the number of bacteria in mucus and in membranes took place much more slowly if the light was partially excluded (as would be the case where membranes were deposited under a bed or in a dark closet). None of the streptococci remained alive as long as three weeks, nor the diphtheria bacilli as long as ten weeks.

T. G. H.

Life Cycles of the Bacteria (Preliminary Communication). F. Löhnis AND N. R. Smith. J. Agr. Res., 1916, **6**, 675–702.

If the writers' claims are true, they will almost revolutionize our conceptions of the morphology of bacteria. They claim that a single species may pass through as many as twelve or fourteen distinct morphological forms, varying from tiny, filterable "gonidia" to large, unorganized masses of "synplasm" formed by the fusion of smaller elements. None of these are dead or attenuated forms (although often described in the past as involution forms), even the "synplasm" being capable of development into organized cells, passing through an intermediate stage in which very small granules appear, called by the writers "regenerative units." The writers distinctly say, however; "We beg to point out that by discussing the life cycles of the bacteria we do not intend to revive any of those unclear theories concerning bacterial polymorphism or pleomorphism. The development of the bacteria is characterized not by the *irregular* occurrence of more or less *abnormal* forms but by the *regular* occurrence of many different forms and stages of growth connected with each other by *constant relations.*"

The writers' most thorough studies have been made on *B. azotobacter.* They show that there are four groups of the different forms of growth of this organism, and that ordinarily any culture shows only one of these groups of growth forms. It is so seldom that a culture develops forms of one of the other groups that separate species have undoubtedly in the past been based upon these morphological differences. The writers have studied a few other bacteria in lesser detail, and have evidence that they also pass through similar life cycles.—H. J. C.

The Inhibiting Action of Certain Spices on the Growth of Microörganisms. Freda M. Bachmann. Jour. Ind. and Eng. Chem., 1916, **8**, 620.

The author has studied the antiseptic action of ground spices, their alcoholic extracts, essential oils and active principles, on pure cultures of mold spores and filaments, and of bacteria. Molds were

represented by species of *Rhizopus, Penicillium, Aspergillus* and *Alternaria* and the bacteria by *B. coli, B. prodigiosus* and *B. subtilis.* The double plate method was used, one half the plate containing plain agar the other half the medium plus the spice to be tested. Tests were made by streaking the plate with sterile water suspensions of the organisms.

Alcoholic extracts of cinnamon, cloves and mustard showed variable degrees of effectiveness. Cloves was most active; mustard not at all. *Rhizopus* was more sensitive than *Penicillium* to cinnamon but less so to cloves. In powder form cinnamon was more effective than cloves, allspice or nutmeg. *Rhizopus* was not affected by cinnamon, other molds and *B. subtilis* were highly sensitive to all spices, except nutmeg; *B. coli* and *B. prodigiosus* were inhibited by allspice but scarcely at all by the others. Cloves and allspice were about equally effective. Cinnamic aldehyde in concentration of 1: 2000 inhibits growth of all molds and bacteria; volatilization inhibits growth even on spicefree half of plate. Eugenol was antiseptic for *Alternaria* and *B. subtilis* only. Of the oils that of allspice was best; effective against *Rhizopus* in 1: 5000, *B. subtilis* 1: 1000; it does not inhibit *B. coli* and *B. prodigiosus.*

In general mold spores were more sensitive than mycelia and both more so than bacteria; *B. subtilis* was most sensitive among latter. Spices in the amounts used in the kitchen are not good preservatives. Large amounts of cinnamon, cloves and allspice do act as preservatives.

I. J. K.

PLANT PATHOLOGY

Observations on Fire Blight in the Yakima Valley, Washington. J. W. HOTSON. Phytopath., 1916, **6**, 288–292.
Leaf infection of Bartlett pears is reported, beginning at the margins. Invasion of the sap wood from twig blight and an abnormal number of fruit infections are also noted.—F. L. S.

Bacteriological Blights of Barley and Certain Other Cereals. L. R. JONES, A. G. JOHNSON AND C. S. READING. Science, 1916, **44**, 432.
The authors describe the causal organism for blights of barley, wheat, rye, spelt, and oats. In all but the latter case the organism seems to be of the same species, a monotrichous rod with a single polar flagellum. It is seed-borne, which accounts for its general distribution. The organism survives on infected seeds for at least two years. More detailed studies will appear at a later date.—C. M. H.

A Bacterial Disease of Western Wheat-Grass, Agropyron smithii. Occurrence of a New Type of Bacterial Disease in America. P. T. O'GARA. Phytopath., 1916, **6**, 341.
A disease of *Agropyron smithii* found in several places in Utah, and similar to the diseases of orchard grass discussed by Rathay is de-

scribed. The affected plants are somewhat dwarfed and have upon their surfaces masses of bacteria which form a yellow ooze which produces layers between the stem and the upper sheath and between the glumes of the inflorescence. The organism does not penetrate into the tissues until some time after it has covered the surface. It is later found in the substomatic chambers and in the intercellular spaces but has not been found occupying the cells. Normal seed are rarely produced. The organism is non-motile and is described by the author as "*Aplanobacter Agropyri* sp. nov." It is a short rod with rounded ends which occurs singly or in pairs and infrequently in chains of four. They are 0.4 to 0.6 x 0.6 to 1.1μ in size. No endospores have been observed. Capsules have been frequently demonstrated. The organisms stain readily with the common stains.

The group number is 212.2223522.—F. L. S.

Some Properties of the Virus of the Mosaic Disease of Tobacco. H. A. ALLARD. J. Agr. Res., 1916, **6**, 649–674.

Several theories have been advanced in the past to explain this disease as of physiological instead of infectious origin. It has been claimed that the oxidases and peroxidases produced by the plant cause the disease, and spread the disease to other plants if inoculated with the sap from diseased plants. The writer gives evidence to disprove this theory. It is true that the virus is not removed from the sap if filtered through a Berkefeld filter; but by submitting the virus to various treatments the writer shows that it may be destroyed by means that do not destroy enzymes, or that the enzymes, on the other hand, may be destroyed by means that do not destroy the virus. The writer concludes: "Since this pathogenic agent is highly infectious and is capable of increasing indefinitely within susceptible plants, there is every reason to believe that it is an ultramicroscopic parasite of some kind."—H. J. C.

PUBLIC HEALTH BACTERIOLOGY

Bubbling Fountain Tests. JANE L. BERRY. Collected Studies, Bureau of Laboratories, New York City, 1914–1915, **8**, 135.

Two bacteriological tests made on a bubbling fountain showed only staphylococci present. When the fountain was smeared with a culture of *B. coli*, considerable flushing did not entirely remove this organism. The danger, if pathogens are present, is evident. The best safeguard for the kind of fountain described is a continuous flow of water.—T. G. H.

Cigar Cutter Tests. JANE L. BERRY. Collected Studies, Bureau of Laboratories, New York City, 1914–1915, **8**, 137.

Of 145 customers purchasing cigars, 74 used the cigar cutter, and of these, 4 first moistened the cigar in the mouth before cutting. Streptococci, staphylococci and spore-bearing rods were easily obtain-

able from the cigar cutter after such procedure. Cigars moistened with *B. pyocyaneus*, when cut, contaminated the cutter, and fresh cigars were in turn contaminated. Of three cigars held in the mouths of different diphtheria patients, one contaminated the cutter with the Klebs-Loeffler bacillus. The author recommends that cigar cutters be abolished.—T. G. H.

Studies on the Use of Brilliant Green and a Modified Endo's Medium in the Isolation of Bacillus Typhosus from Feces. H. C. ROBINSON AND L. F. RETTGER. Jour. Med. Res., 1916, **34**, 363–376.

Using a preliminary enriching medium consisting of peptone water, reaction + 1.0 to phenolphthalein, containing brilliant green in dilutions of 1:60,000 to 1:300,000, and a modified Endo's medium, the authors report successful results in the isolation of typhoid bacilli from feces. The modification of Endo's medium consists in the use of sodium bisulphite instead of the plain sulphite.—H. W. L.

Studies on Diphtheria in Cleveland, I. Diphtheria Carriers. R. G. PERKINS, M. J. MILLER AND H. O. RUSH. Jour. Infect. Diseases, 1916, **18**, 607–617.

A paper having special reference to the problem of shortening the average quarantine period. The points discussed include the incidence of carriers in Cleveland, morphological studies on the organisms found, and the results of the application of the Schick test.—P. B. H.

A Study of the Normal Bacterial Flora of Postage Stamps. R. A. KEILTY AND P. D. McMASTER. Medical Record, 1916, **90**, 153–154.

A bacteriological study of 50 postage stamps obtained from various sources showed the presence of bacteria upon 48 of the 50 stamps.

Methods particularly adapted for the detection of tubercle bacilli *Bacillus tetani*, members of the colon group and diphtherial forms failed to demonstrate any of these organisms. The organisms isolated were, with possibly two exceptions, all non-pathogenic. Micrococci predominated. Other non-pathogenic organisms, molds, *B. subtilis*, etc., were present in only small numbers.—M. W. C.

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